

# BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

## Section B ZOOLOGY

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BULLETIN  
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## CHROMATOPHORE STUDIES

### IV. THE BEHAVIOUR OF THE MELANOPHORES IN THE REGENERATING SKIN OF *DISCOGLOSSUS*

### V. THE PROBLEM OF "EPIDERMISATION" OF THE CORNEA AFTER EXTIRPATION OF THE EYE

H. BYTINSKI-SALZ

*Department of Zoology, University of Tel Aviv*

#### ABSTRACT

The operations were carried out on tadpoles of *Discoglossus pictus* Lat. of 12–15 mm body length.

If a cut is made into the skin dorsal and caudally to the eye, the epidermis covers the wound within a few hours. The epidermal melanophores are passively drawn from the edges of the cut into the regenerating area. After 2 days the mutilated adepidermal melanophores send out regenerating branches or divide and send out cells with a few rather thick filaments, which remain in contact with the old network. No free cells which are richly branched "migrating forms" as occurring under the epidermis of tail regenerates (Bytinski-Salz and Elias 1938), are found. The free cell filaments connect with each other and finally a regenerated net of adepidermal melanophores is established, the meshes of which, however, are much smaller than those of the original net.

The subepidermal melanophores increase greatly in number, by cell divisions of the existing pigment cells, as well as by the precocious formation of pigment in young melanoblasts.

After extirpation of the eyeball, no epidermisation of the remaining cornea occurs. If the surrounding network of adepidermal melanophores is not disturbed, no outgrowth of these melanophores occurs into the cornea. The epidermal melanophores may migrate into the peripheral part of the cornea; the subepidermal melanophores may penetrate into the centre of the cornea, but later degenerate.

After enucleation and excision of the cornea, the regenerated area becomes epidermis. All types of melanophores migrate into the corneal space, the adepidermal melanophores showing the least regenerative and migrating capabilities. In later stages, sense organs and cells of Leydig differentiate. During metamorphosis the mitotic rate is that of epidermis and not of cornea.

After transplantation of the whole eyeball under the skin of the presumptive region of the foreleg, no changes of the epidermis towards transformation into cornea were found.

#### INTRODUCTION

In previous papers (Elias 1936, Bytinski-Salz and Elias 1938, Bytinski-Salz 1938, Elias 1939, 1941, 1942\*) attention was already drawn to the presence of a peculiar pattern of melanophores in the skin of the Discoglossid genera *Discoglossus* and

\* The references of parts IV and V will be given in part VI.

*Bombina*, which seemed to offer the possibility of analyzing further the causes of development of a supercellular structure, which may readily be called a kind of primitive tissue. These adepidermal melanophores, as Elias (1936) named them, form a continuous network over the entire body of the larvae and are firmly attached to the under surface of the skin. This network may consist either of large polygonal meshes as in *Discoglossus* or of a structure, in which the branches of the melanophores cross each other at right angles, as in *Bombina*. But this generic difference in the form of the structure is not of principal importance, since a net of crossing melanophores may also be found in the edge of the tail of *Discoglossus* (Bytinski-Salz and Elias 1938) and a network consisting of polygonal meshes similar to that in *Discoglossus* may be found in a type of *Bombina*, tadpoles with abnormal unpigmented adepidermal melanophores, (Elias 1939). Most important for the origin of this structure was the analysis of its development and dynamics under normal and experimental conditions. We, therefore, studied the normal behaviour of the adepidermal melanophores in *Bombina* from their first appearance as individual cells up to the formation of the network (Bytinski-Salz 1938), the restoration of the adepidermal melanophore network in the regenerating tail of *Discoglossus* (Bytinski-Salz and Elias 1938) and *Bombina* (Bytinski-Salz 1938) and their behaviour in explantation and transplantation experiments.

In this paper the regenerative potentialities of the adepidermal melanophore network was tested further in two ways:

1. in the restorative capacities of the skin after injury
2. in its regenerative capacities in the cornea,
  - a. after enucleation of the eyeball alone,
  - b. in the regenerating cornea after extirpation together with the eye.

The two latter experiments were suggested by the results of Lewis (1905), Fische (1919), Dürken (1913, 1916), Groll (1924), Popoff (1933–1936), etc., who found an “epidermisation” of the cornea with ingrowth of melanophores into the corneal space in the early stages after extirpation of the eye. As the present results are now in complete agreement with those of earlier authors, not only must the other kinds of melanophores be taken into consideration, but also the other types of cell elements present in the larval skin and the larval cornea. Greater importance, however, will be attached to the behaviour of the adepidermal melanophores.

The results represented in this paper are somewhat incomplete, due to the lack of sufficient material for operations. It would have been desirable to follow up the development of the operated larvae further, through all stages of metamorphosis up to the young frog, where the differences between skin and cornea are much more distinct. This would have confirmed the results obtained in earlier stages.

#### MATERIAL AND METHODS

The *Discoglossus* larvae used in the present experiments came from the same sources as described in our previous paper (Bytinski-Salz and Elias 1938). Enucleation of



the eye ball and extirpation of the cornea were carried out on larvae of 12–15 mm length by means of a pair of iridectomy scissors. A cut was made through the skin in a dorsal-lateral direction behind the eye and the entire eyeball including the internal layer of the cornea was lifted by inserting a pair of forceps at the optic nerve and gently removing the eye. In one series, the eye was reimplanted afterwards into a cutaneous pouch behind the pronephros region of the same side. While extirpating the cornea, care was taken to remove at least one, more often 2–3 meshes of the network of adepidermal melanophores surrounding the cornea.

The larvae which had been operated upon were killed at various stages of development up to the middle of metamorphosis and fixed in Bouin, sectioned and stained with hematoxyline Carazzi-eosine or Mallory-azan as described previously (Bytinski-Salz 1938). For the study of melanophores, whole mounts proved to be most useful. Whole larvae, unstained or stained with hematoxyline were dehydrated and cleared in clove oil; afterwards the entire skin of the head including nostrils and corneas of both sides was removed, flattened out and imbedded in Canada balsam. For the study of epithelial boundaries in the regenerating cornea fresh larvae were impregnated with osmic acid-silver nitrate (Hertwig's technique) in direct sunlight, dehydrated, cleared and the skin removed as stated above.

Photographs were taken with the Leitz "Panphot". To increase the contrast in preparations stained with hematoxyline, a blue filter similar to the Wratten contrast filter No. 50 L proved to be most useful.

#### STRUCTURE OF THE NORMAL CORNEA AND THE SURROUNDING SKIN

At the time of operation the cornea of the *Discoglossus* larva is visible as a patch of hyaline tissue over the lens with a diameter of (Figure 1)  $550\mu$ – $800\mu$ . As in all other anurans, it consists of two parts: the cornea externa (pars conjunctivalis) and the cornea interna which are separated by a large intercorneal space filled with liquid. The two layers of epithelial cells of the cornea externa measure about  $12\mu$  in thickness and are free of pigment granules, pigment cells and other cells of secretory type (cells of Leydig). Their inner border is in firm contact with a thin layer of connective tissue, the anterior lamina elastica which forms the continuation of the lamina basalis (cutaneous membrane Grenzlamelle) of the cutis.

During metamorphosis the cornea thickens and develops its characteristic concavity, an adaption which is connected with the transition from subaquatic vision to vision in the air. During these stages numerous cell divisions are found in the corneal epithelium.

The skin surrounding the cornea also consists of two layers, an epidermal epithelium about  $7\mu$  thick and a very thin ( $1\mu$ ) layer of dense connective tissue fibers in a criss-cross formation (Bytinski-Salz 1938) belonging to the cutis. I shall call this layer in the future "Cutaneous Membrane" (Grenzlamelle). The skin distinguishes itself from the larval cornea by the presence of pigment granules in the epidermis, as well as by the presence of a number of different chromatophores (melanophores,



guanophores, erytrophores) and differentiated cell types such as sensory cells and secretory cells.

Unlike the cells of the corneal epithelium, the epithelial cells of the skin always contain small blackish brown pigment granules in their plasma. This is most accentuated in the dorsal part of the body where they form a pigmentary cap at the distal end of the cell, but they can usually be detected also in the epidermis of the lateral sides of the body. At the time of operation Leydig cells are just appearing and are not yet clearly recognizable in whole mounts. Sense buds, belonging to the lateral line system of the head are well developed in the area around the eye. We are here especially interested in a row of sense organs which runs in a cranial-caudal direction ventral to the eye outside of the corneal region. Each organ consists of a circular concentration of cells which are smaller than ordinary epidermal cells and which have more chromatin in the nucleus. The cells in the center of the organ contain more cellular pigment than the surrounding epidermal cells.

Concerning pigment cells, this paper will deal only with the melanophores, of different kinds of which are present in the skin of Discoglossid larvae: epidermal (*EPM*), adepidermal (*ADM*), subepidermal (*SBM*) and subcutaneous (*SCM*) melanophores (Elias 1936, 1937, 1939), (Bytinski-Salz and Elias 1938, Bytinski-Salz 1938). At the stage of operation (from 12–15 mm total length) only the first two and the last types of melanophores are present, while the *SBM* appear in later larval stages.

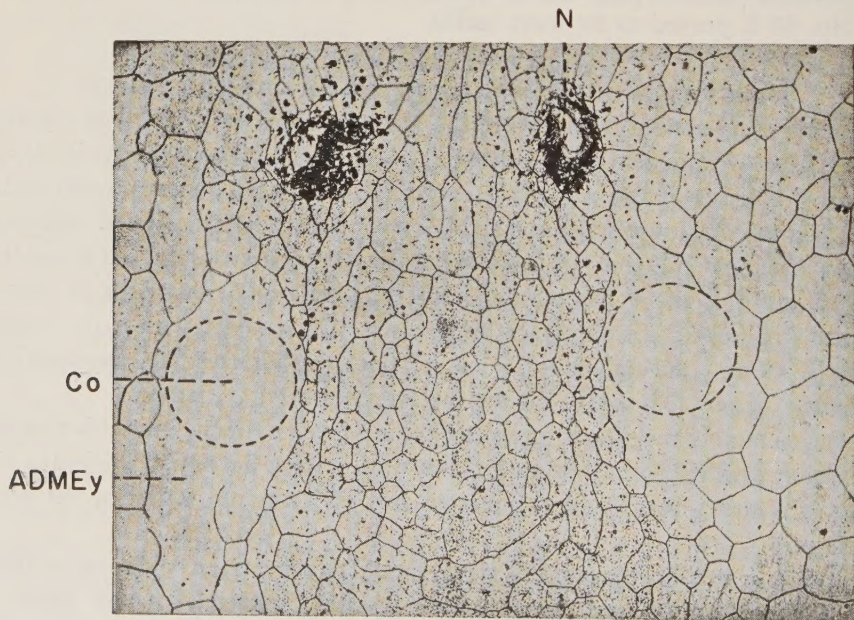


Figure 1

Normal skin of the head of *Discoglossus pictus*, showing the network of adepidermal melanophores, cornea, eye mesh and nostrils; 35 x.  
The circles indicate the limits of the corneal area.



The adepidermal melanophores (*ADM*) form a continuous network of polygonal meshes over the entire body of the tadpole as described in our previous paper (Bytinski-Salz and Elias 1938). Sometimes the meshes are not closed and free end branches of *ADM* may be observed (e.g. Figure 1, in the eye meshes). The cornea lies in a large mesh of the *ADM* network, in the largest ever found on the dorsal and lateral sides of the larval body. But this mesh is larger than the cornea itself and usually more elliptical while the cornea itself is circular. On its ventral border the above mentioned row of sense organs also usually lies within the *ADM* eye mesh; from the dorsal side *EPM* penetrate into its space only up to the border of the cornea. The *ADM* net is found during the entire larval life but degenerates during the later stages of metamorphosis.

The epidermal melanophores (*EPM*) lie in the epidermis either above the cutaneous membrane or between the layers of epidermal cells. Their body is spindle shaped or triangular and branches off into 2-6 primary processes which at the present stage of development branch off dichotomically one or two times. During later larval stages they increase their number of processes and surround the epidermal cells with their network. Each *EPM* may encircle from 20-40 epidermal cells. The *EPM* appear later than the *ADM* and at the stage of operation they are most abundant on the dorsal side of the head down to the eye region. Lateral to the eye they are rather scarce, and the lateral and ventral sides of the body are still free of *EPM*. During further larval development they appear also in these regions, but are always found there in considerably less numbers than on the dorsal side. Their distribution around the eye is very characteristic. From the dorsal side they penetrate into the *ADM* eye mesh first from the dorsal-caudal direction, and later also from a dorsal-cephal direction while ventrally to the *ADM* eye mesh usually 1-3 rows of *ADM* meshes remain free of *EPM*.

The subepidermal melanophores (*SBM*) appear in later stages of the larval life, when the bud of the hind limbs is about 3-4 times as long as is broad. These remain during metamorphosis and the entire adult life, and are one of the most important coloring components of the adult skin (Elias 1936). In early stages their form is similar to that of a connective tissue cell, but shortly after, the cell body branches off and develops into a form with numerous broad but short cell processes each one terminating in a number of fine short rays. The *SBM* appear first in the dorsal region of the skin but are later found distributed over the entire body with the exception of the white parts of the belly.

The subcutaneous melanophores (*SCM*) are the first to appear during embryonic life. They appear at approximately the same time or even a little earlier than the *ADM* and soon begin to migrate over the entire body. They are most conspicuous around the central nervous system, the nostrils (Figure 1) the otic region, the peritoneum and the pericard. As they lie rather deep in the subcutaneous tissue, they are usually not included in whole mounts of the skin and only small numbers of them occasionally remain attached. Their form is similar to that of the *SBM* but more



compact with broader cell body and shorter branches, but transitory forms between *SBM* and *SCM* often occur. They form the main coloring component of the larval skin and persist during metamorphosis and adult life. At these stages the *SCM* of the upper cutaneous layers are so interwoven with the *SBM* that they are scarcely distinguishable, but in the deeper layers they are very distinct.

#### THE BEHAVIOUR OF THE MELANOPHORES IN THE REGENERATION OF THE SKIN

In all experiments of extirpation or transplantation a wound is made in the skin. This cut is closed very quickly and later a regenerative ingrowth of melanophores occurs in this area. In an earlier paper (Bytinski-Salz and Elias 1938) I have already mentioned the extremely limited power of regeneration of the *ADM* net in the region of the throat. Further experiments have now shown that a gradient of regenerative power of the *ADM* net exists, which is highest in the dorsal region and lowest in the belly and the region of the throat. It is probable, that this behaviour is connected with the size of the meshes of the *ADM* net which are smallest in the region of the back and largest on the ventral side, though this may not be the only factor. For comparison with the following chapters it seems desirable to give a short description of the normal regenerative processes in the skin surrounding the eye.

Immediately after a cut is made in the skin, the edges of the wound contract, making the wound broader than the cut itself, but soon the regenerative growth of the epidermal epithelium begins to cover the surface of the wound, a process which is complete within a few hours (for urodels see Lash 1955). The outlines of the epithelial cells on the surface are at first elongated in the direction of tension, somewhat similar to Figure 8a, but they soon attain their normal polygonal shape. The epidermal cells are soon heavily loaded with cell pigment, (Figures 2, 4, 6 *EPP*), which not only appears at the distal cap of the cell but fills the entire cell body. As it is never found in such quantities in the surrounding epidermis, it is evident that this cell pigment arose *do novo* in the regenerating epidermal cells.

At the same time epidermal melanophores appear in the regenerated epithelium. I am very much indebted to Prof. H. Elias who kindly showed me a piece of time-lapse film of a regenerating skin wound in *Bombina*. In this film it can be very clearly seen how the outgrowing epidermal cells cover the wound like a stream of mud in which epidermal melanophores swim like leaves. These *EPM* are sometimes dislocated as required by the boundaries of the flowing epithelial cells, but in general they follow the epithelial stream. From this very impressive picture it is evident that at the first moment the *EPM* are drawn passively into the region of regeneration and do not wander actively into this area.

The *ADM* net may show complete restoration. Already 2 days after the operation, the growth of regenerating branches of the cut melanophores begins. As described in our earlier paper (1938), very slender cell processes appear, which grow out from the mutilated cells (Figure 2). They soon elongate, thicken and penetrate into the regenerated area. Figures 2-4 give pictures of regenerated skin wounds 3, 11 and 21 days



after operation. From Figures 2 and 3 it is evident that this type of regeneration is somewhat different from that described in the regenerating tail (Bytinski-Salz and Elias 1938). The migrating cells show a rather limited number of thick and long branches which do not split up into fine filaments at their ends. These cells are usually connected with the old melanophore net; isolated cells of this kind are found less

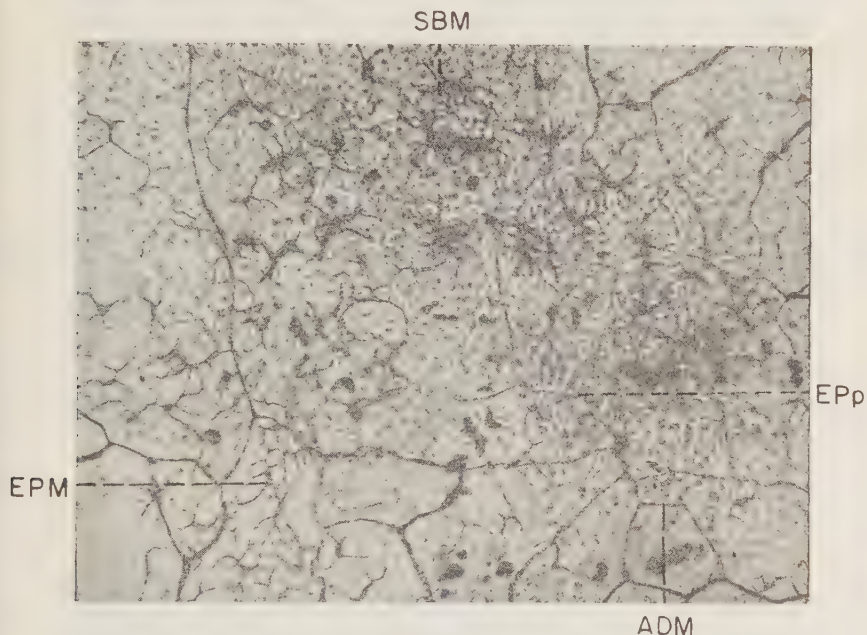


Figure 2

Au 23. Melanophores in the regenerating skin 3 days after operation; Cut obliquely from left above to right below; 160 x.

frequently. Moreover, even if they do occur (Figures 2, 3) they never show the typical form of the "migrating type" as described previously (1938, p. 30-31). They are cells which have migrated from the old net with only a slight change in form. Figure 4 pictures a 21 day old regenerate in which the regenerated ADM net is complete. The meshes are polygonal as in the normal melanophore net, but much smaller. They resemble in every detail the final stages of regeneration in the ADM net in the tail as described for *Discoglossus* (Bytinski-Salz and Elias 1938, p. 33, Figure 35). The first and last stages of the regenerating ADM net in the skin are the same type of regeneration found in the regenerating tail but the intermediate stages are different.

SBM are already present in large numbers 2-3 days after operation. They are found in all stages from complete expansion to complete contraction, in other parts of the body as well. Their number depends upon the number present in the surrounding tissue. Some tadpoles with relatively few SBM in the skin show also only a

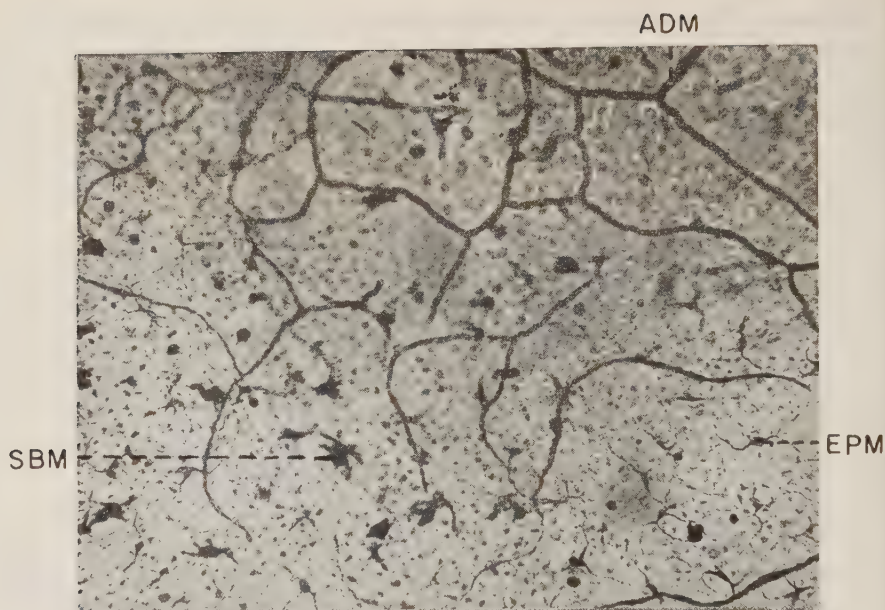


Figure 3

Au 26. Melanophores in the regenerating skin 11 days after operation;. Cut horizontally in the lower half of the picture; 160 x

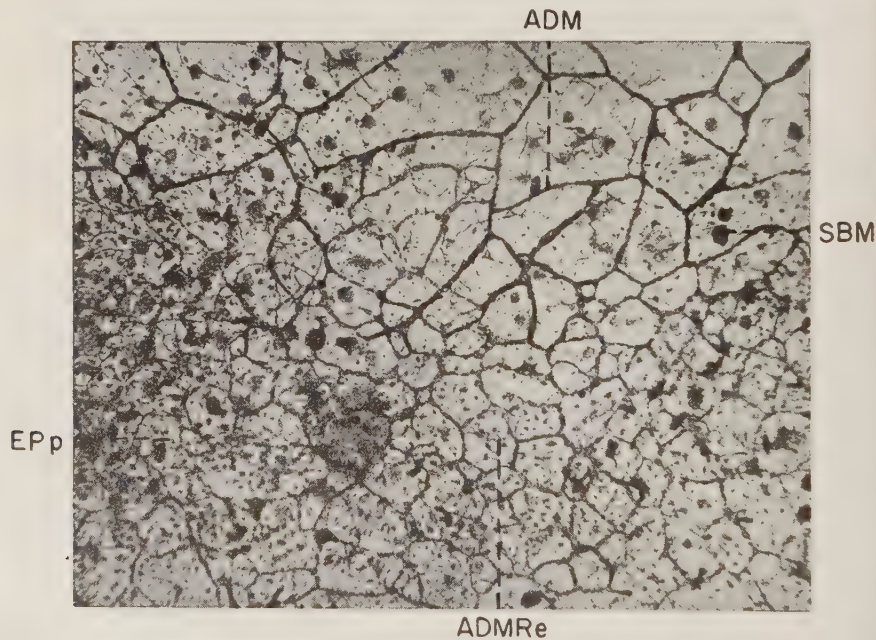


Figure 4

Au 41. Regenerated net of adepidermal melanophores in the regenerating skin 21 days after operation;. Cut horizontally in the lower part of the picture; 160 x.



small number in the region of the cut; other larvae with more melanophores show also more *SBM* in the wound region. The number of *SBM* increases rapidly, and after 3–6 days their number is so large, that in some cases, they may form a mosaic. In a 9 day old regenerate (Au 8, Figure 6) the number of *SBM* in one observation

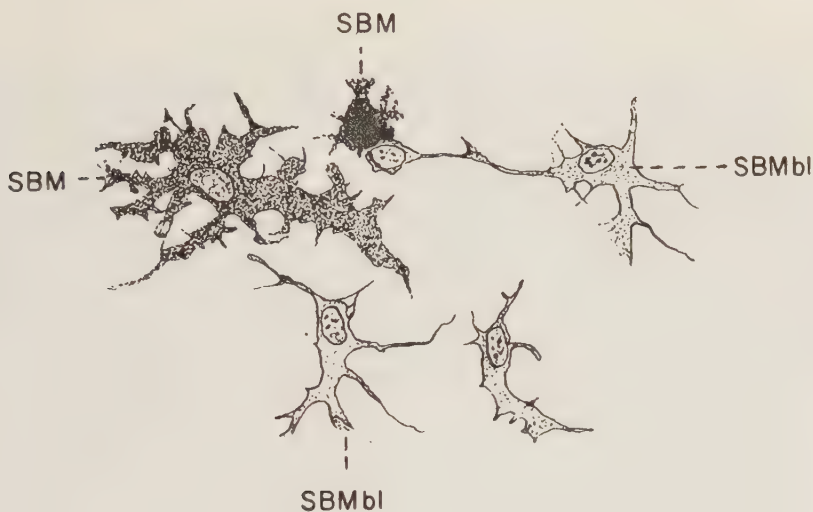


Figure 5

Au 23. 4 developing subepidermal melanoblasts and 2 subepidermal melanophores in the regenerating skin 3 days after operation; 520 x.

field was 64, while on the corresponding normal side only 15 *SBM* were counted. Since in 3–6 day old regenerates the *SBM* are often found lying together in pairs, it seems evident that cell division plays an important part in this increase in the number of melanophores. But another source may also contribute to this increase. It must be borne in mind, that the *SBM* begin to appear shortly after the stage of operation, and also normally their number will increase during larval life. As was already pointed out for *Bombina* (Elias 1936, 1937), the *SBM* evolve from a mesenchyme-cell-like type of melanoblasts which are devoid of pigment granules. During further development the cell body flattens out, its branches broaden, the size of the nucleus increases and pigment granules develop. This type of developing *SBM* is also found in the regenerating area. Figure 5 shows four of these developing melanoblasts at different stages of pigment formation together with one expanded and one contracted *SBM*. To the right is a cell shortly after division, indicating that the *SBM* are able to divide also in their melanoblast stages. Similar stages of development of the *SBM* are also found in other parts of the skin but there they are much

less frequent than in the regenerated region. It seems possible therefore, that the stimulus of a wound in the skin accelerates the ingrowth of melanoblasts and perhaps hastens the pigment formation in the existing premelanophores. If we remember that a large increase of pigmentation is also found in the epithelial cells in the regene-

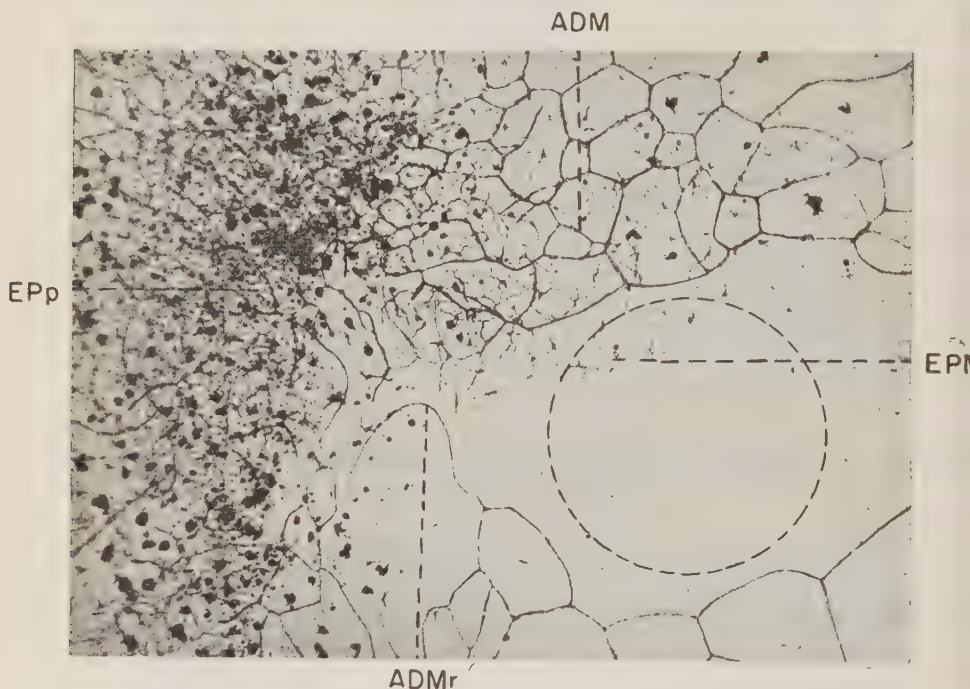


Figure 6

Au 8. Ingrowth of melanophores into the cornea after extirpation of the eyeball; 9 days after operation; Cut almost vertically in the left quarter of the picture; 80 x  
The circle indicates the limits of the corneal area.

rated area it is by no means inconceivable that the same process may also accelerate pigment formation in the melanoblasts which makes them more easily detectable afterwards for microscopic observation.

In connection with this, a paper by Niu and Twitty (1950) must be mentioned, in which the authors claim that in *Triturus* (Urodela) mesenchymal cells of the macrophage type are able to engulf pigment particles arising from degenerating melanophores and are later able to transform themselves into typical epidermal melanophores. I think that this assumption is not valid in our case for the following reasons: the process of macrophage-melanophore transformation is a slow process requiring a



least 8–10 days in *Triturus*, while in our experiments a large increase of *SBM* can already be observed 2–3 days after the operation; the amount of free melanin arising from the cut and degenerating melanophore branches is too small to explain the considerable increase of pigment in the supernumerary *SBM*; no young typically branched *SBM* were observed with ingested pigment particles.

#### THE BEHAVIOUR OF THE MELANOPHORES AFTER EXTIRPATION AND TRANSPLANATION OF THE EYE AND REMOVAL OF THE CORNEA

The following series of experiments were carried out. In each case the right eye was operated on and the left eye kept for control.

##### A. EXTIRPATION EXPERIMENTS

1. *Enucleation of the eyeball only* .. .. . 15 cases
2. *Enucleation of the eyeball and extirpation of the overlying cornea* .. 25 cases
3. *Enucleation of the eyeball and extirpation of the regenerated cornea for a second time* .. .. . 10 cases
4. *Enucleation of eyeball and extirpation of the cornea 48 days later*.. 10 cases

##### B. TRANSPLANTATION EXPERIMENTS

5. *Transplantation of the enucleated eyeball under the skin in the region of the pronephros* .. .. . 20 cases

##### 1. *The enucleation of the eyeball.*

After enucleation of the eyeball by means of a cut dorsally and caudally to the eye, the wound heals quickly but the area around the cut is recognizable also afterwards by the large amount of pigment granules in the epidermis and the increased number of subcutaneous melanophores (Figure 6). The first reaction of the cornea in the absence of the eye is an enlarged immigration of *EPM* from dorsal, cephal and cephaloventral regions. Later on, after the appearance of the *SBM*, these also begin to migrate into the corneal space. The eye mesh of the *ADM* net does not show any signs of outgrowth as long as it remains untouched by the operation. Two cases will demonstrate the behavior of the melanophores:

Au 8. Whole mount 9 days after enucleation (Figure 6). On the unoperated (control) side 8 *EPM* are found in the dorsal and dorsal-caudal part of the *ADM* eye mesh. They do not penetrate into the corneal area. On the operated side 31 *EPM* are found within the *ADM* eye mesh lying mostly dorsal and dorsal-caudal, with 4 penetrating also from rostral. From dorsal they reach the upper part of the corneal area. The *ADM* net shows decided regeneration around the region of the cut, but no *ADM* penetrate into the space of the eye mesh, though the latter is not complete at its caudal border. One *SBM* is found inside of the eye mesh at its rostral edge, none on the control side.

Au 12. Whole mount 89 days after enucleation (Figure 7). The size of the *ADM* eye meshes on both sides is very unequal, the one on the control side being much larger.



Figure 7

Au 12. Ingrowth of melanophores into the cornea 89 days after enucleation of the eyeball; degenerating subepidermal melanophores in the cornea; 200 x.  
The part of the circle indicates the limits of the corneal area.

Therefore, 77 *EPM* are found there and not more than 80 *EPM* on the operated side. But the ingrowth from rostral is very evident, since 16 *EPM* were counted there compared to 3 on the normal side. On the dorsal side, the *EPM* reach the edge of the corneal space but do not penetrate far into it. The *ADM* eye mesh is closed and does not show any signs of outgrowth into the corneal area. *SBM* are abundant in the dorsal skin between the eyes and penetrate on the control side into the *ADM* eye mesh but do not reach the cornea. On the operated side they penetrate from the dorsal part of the eye mesh into the corneal region and a few scattered *SBM* are also found ventral to the center of the cornea. All of the *SBM* show distinct signs of degeneration (*SBMd*). The cell nucleus stands out most clearly, as no pigment granules are found above it. The cell branches are retracted, and isolated pigment granules, sometimes arranged in rows, are scattered around the cell body, indicating the position of degenerated processes. The further these melanophores penetrate into the cornea the more degenerate they appear. But it is evident through a continuous series of transitory stages between normal *SBM* and these degenerated pigment cells that they are derived from *SBM*. It seems, therefore, that the tissue below the normal cornea is not a suitable living space for the *SBM*. They immigrate into this region but later on degenerate.

This series of experiments demonstrates, that after enucleation of the eyeball *EPM* penetrate into the peripheral regions of the cornea but not to the centre. The *ADM* net, if left undisturbed shows no signs of ingrowth at all. The *SBM* penetrate from the dorsal region into the cornea and may even reach the centre, but later on they degenerate. There can be no question of "epidermisation" of the cornea after extirpation of the eye only, at least not during larval life. The cornea remains always



clear without the appearance of pigment granules in the corneal epithelium and also without melanophores even as long as 89 days after enucleation, i.e. up to the beginning of metamorphosis.

# 1. *Enucleation of the eyeball and single extirpation of the cornea*

In this series of experiments, the entire cornea was extirpated and the eyeball was removed. After the operation, the outgrowing epithelium covers the wound in less than 12 hours. After 18 hours the aspect of the regenerated epithelium (osmic acid-silver nitrate impregnation) is as in Figure 8a. A large number of epithelial cells are elongated in the direction of the tension (in Figure 8a from left above to right below). During the next few days, cell divisions occur mainly in these elongated cells

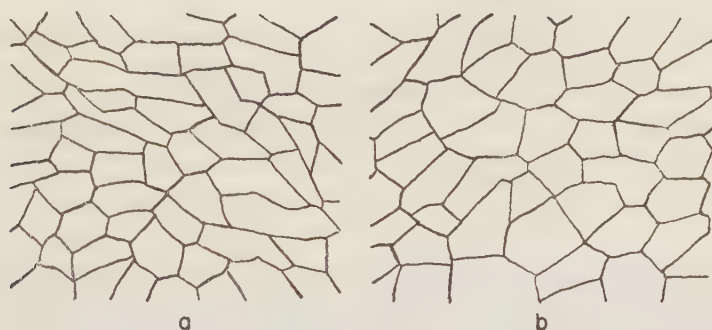


Figure 8

Figure 21. Cell boundaries (silver impregnation) of a. the regenerated corneal epithelium and b. the normal corneal epithelium. 18 hours after extirpation of the eye and the cornea, 300 x.

and finally they assume the appearance of a normal epithelium with polygonal cells (Figure 8b). Already 3 days after operation, the corneal region has reached the appearance of epidermis. Pigmentation of the epithelial cells, *EPM* and *SBM* are present even in the centre of the cornea, and abnormally placed sense organs may be observed in different parts of the corneal area. The *ADM* net sends cell processes into the regenerating area, though they do not reach its centre. Somewhat later, a further increase of *EPM* and *SBM* may be observed, but the *ADM* do not penetrate further into the corneal space. The description of a few cases will demonstrate this behaviour.

Au 22. whole mount 3 days after operation. The region of the cut shows a large increase of pigment in the epidermal cells. *EPM* and *SBM* present in large numbers, the latter often maximally contracted. The *ADM* net shows regenerating branches (*ADM*). The epidermal cells in the regenerated cornea show increased pigmentation; sometimes the pigment granules are fused into little specks. *EPM* present all over the extirpated area; *SBM* absent in its centre but fairly common in the peripheral parts. They

are especially abundant on the ventral border of the eye mesh and penetrate from there into the lower part of the regenerated area. Three abnormally placed sense organs (*SB*) are present at the periphery of the cornea. The *ADM* net shows regenerating branches which penetrate from a ventral caudal direction into the corneal space. Figure 9 shows, on the left, a typical regenerating *ADM* which sends out a fine thread-like regenerate (*ADM*) from the cut end. In the centre is a group of young *ADM* (*ADMm*) which has migrated from the left into the lateral part of the cornea and which attempts to form a new network. Their form is very similar to that observed in the regenerating tail but much unlike that observed in the regenerating skin of t

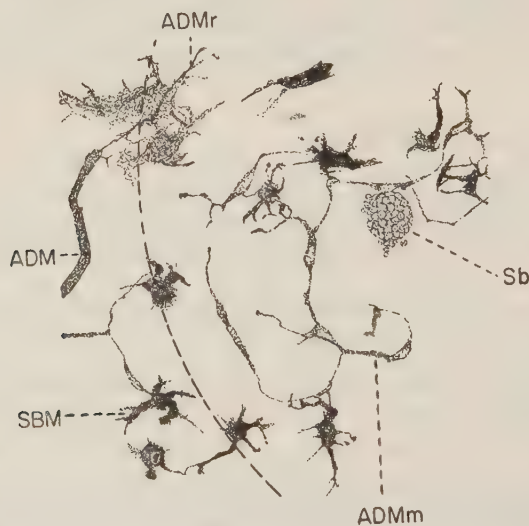


Figure 9

Au 22. Ingrowth of melanophores and sense organs into the corneal space 3 days after extirpation of the eye and the cornea; 145 x.

The part of the circle indicates the limits of the corneal area.

trunk. They are typical "migration forms" with many slender and branched processes.

A blood capillary runs straight across the centre of the cornea.

Au 26. Whole mount 11 days after operation. The cut shows a rather complex regenerated net of *ADM* (Figure 3) from which branches penetrate into the opercular region (Figure 10). From the ventral side, *ADM* penetrate almost to the middle of the corneal area, but with the exception of one branched cell, only undivided processes of the remaining *ADM* net grow out. *EPM* are abundant over the entire area. *SB* penetrate from a dorsal and dorsal-lateral direction but do not reach the centre. Most of them are maximally contracted. There are 6 abnormally placed sense organs (*SB*) in the extirpated area, 3 at the dorsal edge, 2 directly in the centre and one at its ventral border.

Au 1. Whole mount 22 days after operation (Figure 11). The regenerated epithelium



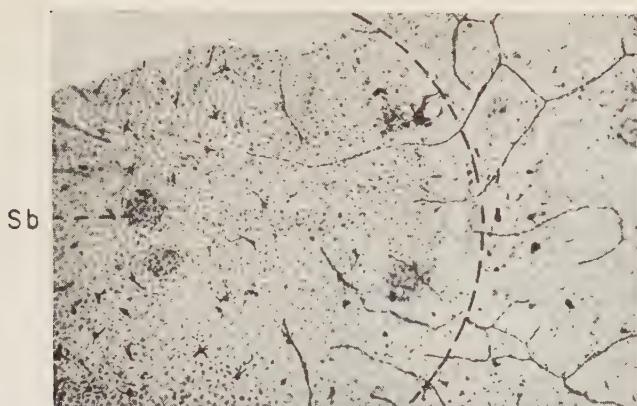


Figure 10

Au 26. Ingrowth of melanophores and sense organs into the corneal space, 11 days after extirpation of the eye and the cornea; Hematoxylene stain; 95 x.

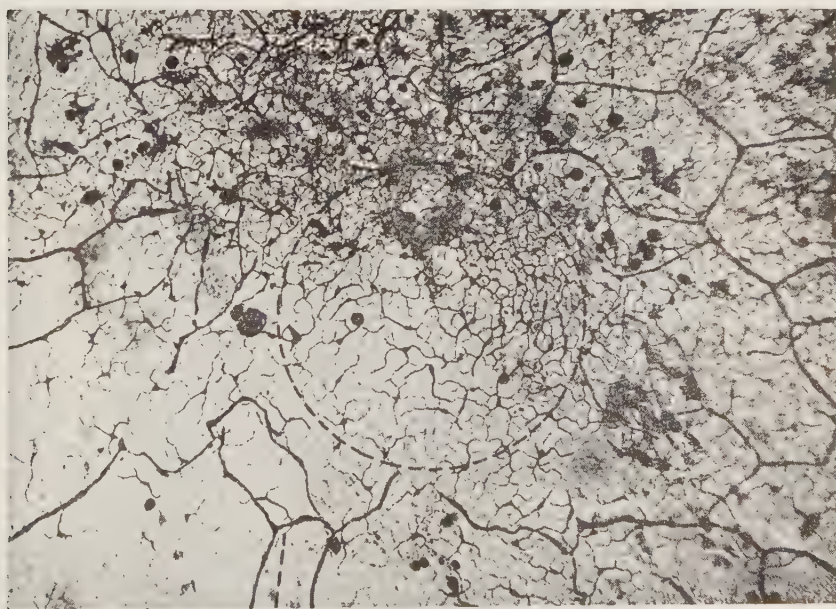
The part of the circle indicates the limits of the corneal area.

cells show an increase in pigment granules (*EPp*) in the dorsal region but are almost free of them in the ventral area. *EPM* are distributed in large numbers over the entire extirpated space. *SBM* are maximally expanded and migrate from dorsal and

**EPp**

**SBM**

**G**



**ADM**

Figure 11

Au 1. Ingrowth of melanophores and guanophores into the corneal space, 22 days after extirpation of the eye and the cornea; 95 x.

The circle indicates the limit of the corneal area.

rostral directions but do not reach the centre of the corneal area. There is an *ADM* net with very few regenerating branches but none of them penetrate into the corneal space. Globular guanophores (*G*) are abundant at the dorsal part, two of them also present in the central region of the cornea.

### 3. *Enucleation of the eyeball and double extirpation of the cornea.*

Since previous experiments have shown that a rather limited migration of melanophores, especially *ADM*, occurs into the regenerating area, it was thought that perhaps a second regeneration may achieve better results. For this purpose the epithelium of the corneal region was allowed to regenerate for 21 days after enucleation and extirpation, and then removed for a second time. The larvae were raised for a further 18–34 days after the second operation up to the beginning of metamorphosis. However, while *EPM* and *SBM* readily penetrated into the regenerating area, regeneration and ingrowth of the *ADM* were still lower than in younger stages; they did not penetrate at all into the corneal space.

Au 42. Second extirpation of the cornea 21 days after the first; fixed at the beginning of metamorphosis, 55 days after the first operation; whole mount.

Figure 12 gives a picture of the centre of the regenerated area. The epithelium is almost clear. *EPM* present, mostly contracted and in stages of degeneration (*EPMd*).

*SBM* present in large numbers, all of them contracted. The regenerated *ADM* do not even reach the border of the corneal area.

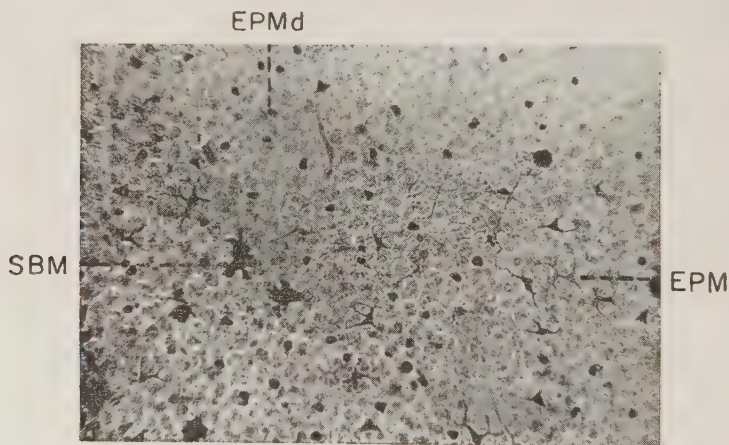


Figure 12

Au 42. Melanophores in the regenerated corneal area after extirpation of the eye and the cornea and renewed extirpation of the regenerated cornea; fixed 55 days after the first operation at the beginning of metamorphosis; Hematoxyline stain; 160 x.

### 4. *Enucleation of the eyeball and delayed extirpation of the cornea.*

Since the possibility that perhaps a secretory influence from degenerating tissues in the orbit may inhibit the ingrowth of melanophores into the regenerating corneal



space was not excluded, the cornea was left intact for 48 days after enucleation of the eyeball, then extirpated and allowed to regenerate for 31–45 days, in some cases up to the middle of metamorphosis. The results agree well with those of series 1 (enucleation and extirpation of the cornea at the same time). In general, *EPM* and *SBM* are present in the centre of the extirpated area, only in one case (Au 46) is this region completely void of *SBM*. The *ADM* show a rather limited regenerative power, even less than in the cases of series 1. Only in one case, which will be described below, a somewhat greater degree of regeneration occurred, which, however, did not lead to a continuous *ADM* network over the entire regenerated area.

Au 53. Extirpation of the cornea 48 days after enucleation. Killed 45 days afterwards in a stage of advanced metamorphosis (fore limbs free); whole mount.

The normal cornea is completely clear, concave, protruded and shows a large number of dividing epithelial cells (Figure 13a, Com, Table I). Above the normal eye

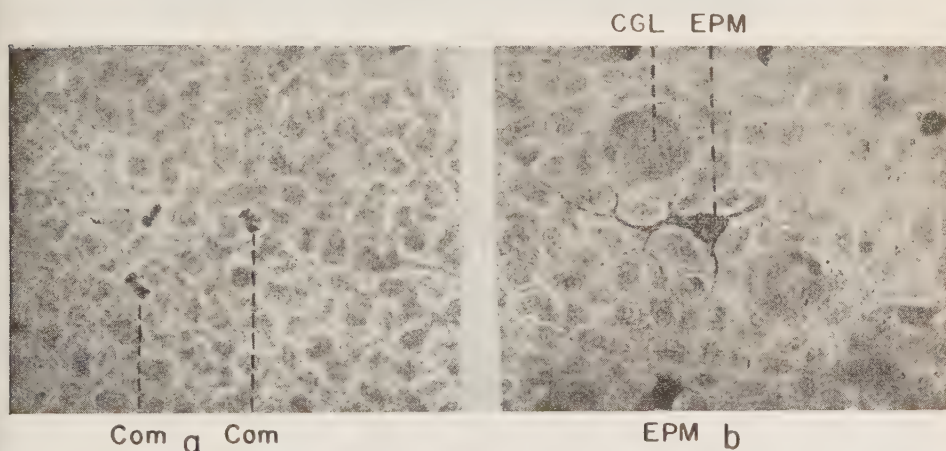


Figure 13

Au 53 a. Normal cornea at the middle of metamorphosis with 3 epithelial cells in division. b. Extirpation of the cornea 48 days after extirpation of the eye; fixed 45 days afterwards. Regenerated epidermis in the corneal area with epidermal melanophores and developing cutaneous glands; Hematoxyline stain; 500 x.

the *SBM* surround the developing cutaneous glands. The extirpated area on the other side gives the appearance as seen in Figures 13b, 14, 15. The epithelial cells show a small amount of pigment granules. Developing cutaneous glands (Figure 13b *CGL*) are abundant over the entire corneal region. *EPM* (Figure 13b) are evenly distributed over the entire area. In the centre, they are mostly contracted but gradually expand towards the dorsal-caudal edge. *SBM* (Figure 14) are found in large numbers, especially in the ventral half; they are maximally expanded. The *ADM* net shows regenerative outgrowth, but reaches the corneal area only from the ventral-rostral side. Here an abortive *ADM* net is found with meshes smaller than normal (Figure 15) but it does not penetrate into the corneal space. In the central region, several large

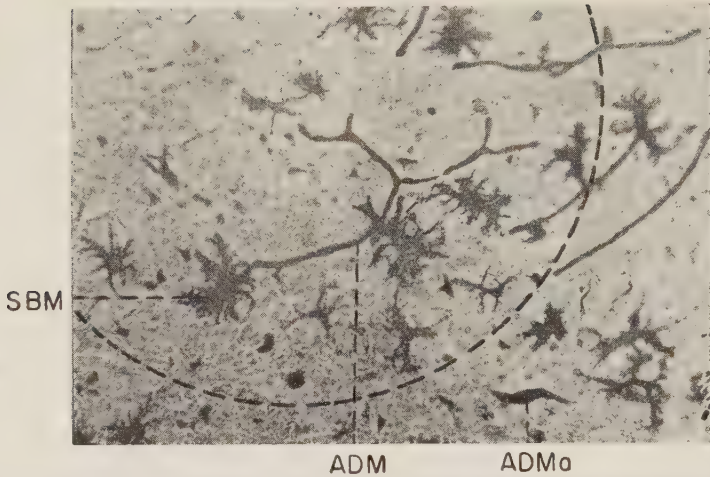


Figure 14

Au 53. Regenerated epidermis with adeipidermal and subepidermal melanophores growing into the space of the extirpated cornea; Hematoxyline stain; 160 x.



Figure 15

Au 53. Regenerated net of adeipidermal melanophores at the beginning of their physiological degeneration during metamorphosis at the edge of the regenerated corneal area; 165 x.  
The part of the circle indicates the limits of the corneal area.

branched *ADM* (Figure 14) are found with thick and stout processes (Figure 14). They seem to be in the first stages of their normal physiological degeneration, which occurs during the middle of metamorphosis. In the regenerated area, but more caudal to the corneal region lies a group of *ADM* which show this degeneration to a much higher degree (Figure 14 *ADMd*).



## GENERAL CONSIDERATIONS ON THE EXTIRPATION OF THE CORNEA

The results of simple or double cornea extirpation, simultaneously or after the nucleation of the eyeball are so similar, that they may be considered together. The change of the regenerated cornea into epidermis is a complex process, consisting of a number of individual elements each of which may be found or may be absent in the regenerated corneal space. The elements necessary for complete epidermisation which will be considered here are:

## DURING LARVAL LIFE:

1. Pigmentation of the epithelial cells;
2. Presence of sense organs;
3. Presence of *EPM*;
4. Presence of *ADM*;
5. Presence of *SBM*; to which are added:

## DURING METAMORPHOSIS:

6. The development of cutaneous glands;
7. The lower thickness of the epidermal epithelium compared with the epithelium of the cornea;
8. The absence of cell divisions in the epidermal epithelial cells;
9. The increased growth in the cutaneous membrane and
10. The absence of a convexity as formed in the cornea ("Brille").

Each element for epidermisation may or may not occur in the centre of the corneal area and therefore a large amount of variation may occur, ranging from complete epidermisation to the presence of a clear, hyaline, cornea-like space. Both extremes occur, though they are much rarer than intermediate stages.

1. The increase of pigment in the regenerating epithelium is very pronounced in most of the cases. In maximal cases, it is as large as in the surrounding epidermis but never shows the enormous increase as found in the epidermis covering wounds in the skin (P. 17)
2. The sense organs, which form part of the regenerating epithelium, are drawn passively into the corneal space. They belong mainly to the row of sense organs, which normally lie below the eye, and may be found even in the centre of the corneal area (Figure 10).
3. The *EPM* are also drawn passively into the regenerated space in the same way as described for a regenerating wound (P. 17). However, they have also the potentiality to migrate actively into the cornea if only the eye is extirpated and the cornea left intact (Figures 6, 7).
4. The *ADM* show the least potentiality to grow into the regenerating region and rarely reach the centre of the area. For the peripheral parts, their type of outgrowth from the old *ADM* net is similar to the type of regeneration as described in the regenerating skin (P. 18) but in later stages typical migrating melanophores are found towards the centre of the corneal region (Figure 9), which are identical with the "migrating cell type" as described in the outgrowing

*ADM* net in the regenerating tail. A complete regenerated meshwork of *ADM* as found in the regenerated skin or tail, was, however, never observed, thus demonstrating the very limited regenerative potencies of the *ADM* in the corneal region. Also in the experiments with double or delayed extirpation of the corneal region, no better results were achieved in regard to the formation of an *ADM* net. It seems probable, therefore, that if some greater activation of the regenerative power of the *ADM* net would have been caused by this type of double operation, its result would have been compensated by the older age of the larvae (22 days older at the time of the second operation), which results in a decrease of the regenerative power of the *ADM*.

5. The *SBM* grow actively from the edge of the extirpated region into the corneal space and in a large number of cases they are found in its centre (Figure 14) though not as often as the *EPM*. Their number is always decreased and they never fill out the regenerated area as uniformly as in the normal skin.
6. The presence of cutaneous glands in the corneal region was observed in all 3 cases which were brought up to metamorphosis (Figure 13 b).
7. At the beginning of metamorphosis, the regenerated epithelium has, with a height of 18–22 $\mu$ , about the same height as the normal adjacent epithelium (20–25 $\mu$ ), while the differentiating corneal epithelium, with 35 $\mu$ , is much thicker.
8. Also, the number of mitoses of the epithelial cells clearly indicates that there is no increase of mitotic activity in the regenerated epithelium while this activity is very evident in the differentiating cornea (Figure 13a). Measurements were made with the aid of a net of 100 squares, inserted into the ocular, each side of the square measuring 23.5 $\mu$ . The number of resting nuclei was estimated in each case by the average of 100 squares, and the number of dividing nuclei (Prophase-Telophase stages) measured in an area of 400 squares = 470 $\mu$  x 470 $\mu$  in the centre of the cornea. The mitotic indices for the three cases in metamorphosis are tabulated in Table I.

TABLE I  
Mitotic indices of the cornea and the regenerated corneal area during metamorphosis

No.	Operation	fixed	resting nuclei of the epithelium per 100 $\square$ = 235 $\mu$ x 235 $\mu$		dividing nuclei per 400 $\square$ = 470 $\mu$ x 470 $\mu$		Percent of mitoses	
			cornea	regener. area	cornea	regener. area	cornea	regener. area
Au 42	double cornea extirpation	beginning of metamorph.	535	430	28	4	1.31 %	0.232 %
Au 46	cornea extirpation 48 days after enucleation	beginning of metamorph.	535	365	15	3	0.701 %	0.201 %
Au 53	cornea extirpation 48 days after enucleation	forelimbs free	505	516	28	2	1.33 %	0.097 %



This clearly demonstrates, that the mitotic activity in the regenerated epithelium is from 3.5 to 13 times smaller than that of the cornea undergoing metamorphosis and agrees well with that of the epidermis which is also almost nil.

9. In two cases sectioned during metamorphosis and also in some full grown tadpoles of premetamorphosis age, a decided thickening of the cutaneous membrane was observed. At the begin of metamorphosis, the layer measured about  $6-7\mu$  in the normal and regenerated skin while the developing anterior elastic lamina of the cornea measured only  $4\mu$ .
10. In three whole mounts of cases killed during metamorphosis, the normal cornea is clearly protruded to form a convex watch glass-like shape, while the skin of the operated side remains flat. This is especially evident in later stages (Au 46) and is in agreement with the lack of mitotic activity in this area.

It is evident, therefore, that complete epidermisation may occur in the regenerating issue lying above the extirpated eyeball, even if during larval life no complete regeneration of the *ADM* net occurs. Since this *ADM* net degenerates during metamorphosis and no traces can be found in the young frog, epidermisation of the regenerated skin may become complete at the end of metamorphosis.

##### 5. *Transplantation of the enucleated eyeball under the skin in the region of the pronephros.*

In 20 tadpoles of 12-15 mm body length the enucleated eyeball was inserted into a cutaneous pouch directly under the skin in the region of the pronephros. A number of whole-mounts proved to be very unsatisfactory for the histological study of the melanophores in the lens region of the transplanted eye, as the black pigment of the eyeball obscures every overlying structure. In 4 out of 6 sectioned cases the internal cornea of the grafted eye either touches the overlying skin of the host tissue or is sufficiently near it to exert its influence. Since all 4 cases show the same negative result, it seems sufficient to describe one of the oldest ones which, however, shows interesting details in other respects.

Au 30. Fixed 40 days after operation; before killing placed for half an hour in 0.1% cocaine.

The grafted eye lies in the mesenchymal space between the kidney and the forelimb. It is well developed in all its parts with a globular lens (Figure 16a). The optic nerve grows between the pigment epithelium of the retina and the choroid in a dorsal direction and loses itself near the edge of the iris. The outgrowing limb bud (*Lb*) strongly presses the whole eye against the skin of the host (Figure 16b), thereby bringing the internal cornea (*Ci*) in close contact with the cutaneous membrane tissue of the host (Figure 16, 17). All conditions are, therefore, given so that the grafted eye may exert its influence upon the skin of the host, but no such influence could be found. The epidermal cells do not contain pigment granules, but these are always present in the skin of this body region. *EPM* and *ADM* are abundant in the space overlying the lens. A count was made of the melanophores in a circular region of the skin of the diameter of the lens ( $=430\mu$ ). *EPM* were found in 36 and *ADM* in 30 out

of 43 sections. Figure 17a,b gives two sections of the host skin (*C-Ep-Cm*) and the internal cornea (*Ci*) of the graft attached to it. The two types of melanophores *EPM* and *ADM* with their nucleus (*ADMn*) are clearly visible.

*SBM* are completely wanting in this region though they are abundant in the su

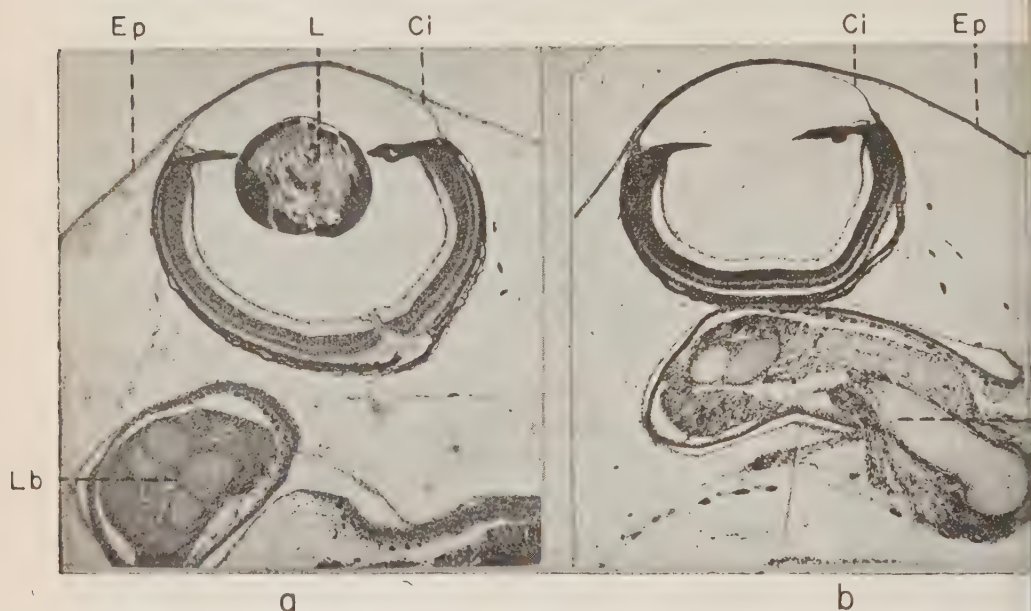


Figure 16

Au 45. Transplantation of the eye under the skin of the body, 40 days after transplantation. a. median section through the grafted eye; b. section showing the outgrowing limb bud pressing the eye against the skin of the host; Hematoxyline-eosin stain; 45 x.

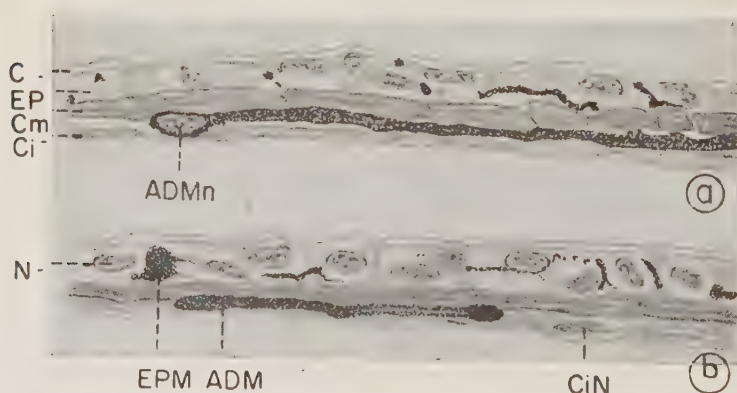


Figure 17

Au 45. a. and b. Sections through the distal part of the eye, showing the internal cornea of the graft and the overlying skin of the host tissue with normal epidermal and adeidermal melanophores; Hematoxyline-eosin stain; 700 x.



ounding skin. This may be explained by the theory that the internal cornea has attached itself already prior to the appearance of the *SBM* and barred the way for their migration.

These experiments, therefore, demonstrate that in 12–15 mm long tadpoles within the time of 40 days, the eyedoos not exert an influence strong enough to convert the overlying skin into a eye does not exer. an influence strong enough within the time of 40 days, the cornea. Whether an influence in earlier stages or of longer duration would achieve this alteration in *Discoglossus*, must be a matter of further experiments.

## EXPLANATION OF SYMBOLS

<i>ADM</i>	= adepidermal Melanophores
<i>ADMd</i>	= degenerating adepidermal Melanophores
<i>ADMEy</i>	= adepidermal eye mesh
<i>ADMm</i>	= migrating adepidermal Melanophores
<i>ADMn</i>	= nucleus of adepidermal Melanophore
<i>ADMr</i>	= regenerating adepidermal Melanophore
<i>ADMRe</i>	= net of regenerated adepidermal Melanophores
<i>C</i>	= epidermal cuticle
<i>CGI</i>	= cutaneous gland
<i>CI</i>	= internal cornea
<i>CIN</i>	= nucleus of cells from the internal cornea
<i>Cm</i>	= cutaneous membrane (Grenzlamelle)
<i>Co</i>	= corneal epithelium
<i>Com</i>	= mitosis in the corneal epithelium
<i>Ep</i>	= epidermis
<i>EPM</i>	= epidermal Melanophores
<i>EPMd</i>	= degenerating epidermal Melanophores
<i>EPN</i>	= nucleus of epidermis cells
<i>EPp</i>	= pigment in the epidermis cells
<i>G</i>	= guanophore
<i>Lb</i>	= limb bud
<i>N</i>	= nostril
<i>Sb</i>	= sense organ
<i>SBM</i>	= subepidermal Melanophores
<i>SBMbl</i>	= subepidermal Melanoblasts
<i>SBMd</i>	= degenerating subepidermal Melanophores

# CHROMATOPHORE STUDIES

## VI. THE BEHAVIOUR OF THE MELANOPHORES IN THE DEVELOPING LIMB BUD OF *DISCOGLOSSUS PICTUS*

H. BYTINSKI-SALZ

*Department of Zoology, University of Tel Aviv*

### ABSTRACT

The skin region covering the outgrowing limb of the tadpoles of *Discoglossus pictus* contains the same network of adepidermal melanophores as that covering the remainder of the body. The outgrowing limb bud presses against these melanophores, causing partial degeneration of their filaments. The network loosened in this way is then drawn out passively over the outgrowing bud. Only at a later stage (bud about 0.6 mm long), the first adepidermal melanophores of the "migrating type" are observed, as found also in the regenerating tail. They multiply and in later stages move distally till the tips of the toes. They repeatedly try to establish new melanophore nets consisting of smaller meshes than those found in the original net, but never succeed to cover the entire limb with a continuous net. This shows that the adepidermal melanophores retain their proper rate of division and are not, or at least to a minor degree, stimulated by the enormous increase of the skin area of the outgrowing limb.

The form and behaviour of the other types of melanophores is described. Their appearance in the outgrowing limb is the same as in the dorsal region of the body, only that on the ventral side all of them appear later. Their sequence is: first subcutaneous, adepidermal, epidermal and finally subepidermal melanophores.

### INTRODUCTION

The network of adepidermal melanophores in the skin of *Discoglossus pictus* exists in a state of dynamic equilibrium. That is, while individual cells may divide, connections between different cells may be severed and new connections may be formed. During the growth of the tadpole, the network itself always remains without changing its general form of polygonal meshes. Only in later larval life, this synchronous growth is disturbed in the areas surrounding the outgrowing limb buds. One must bear in mind that in about 10 days time the skin area covering the outgrowing limb bud is increased several hundred times by the heterogeneous growth of the mesenchymal limb bud. While the epidermal cells of the skin adapt themselves well to this stimulus and respond by an increase in the number of cell divisions, the question arises as to the way in which the melanophores, especially the highly specialized adepidermal ones, are capable of following the stimuli of the quickly increasing skin area. In this paper, therefore, the behaviour of the melanophores in the outgrowing hind limb bud is studied in comparison with those of the regenerating tail (Bytinski-Salz and Eliasson, 1938), where similar conditions of rapid growth occur.

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## MATERIAL AND METHODS

The first rudiments of the hind limb bud become visible in *Discoglossus* larvae of about 18–22 mm total length. From this stage on till the beginning of metamorphosis, the limb bud area with its overlaying skin, or in later stages the young limb, is cut out after fixation with Bouin's fluid, dehydrated and cleared in clove oil. Drawings of the melanophore pattern were made with the aid of the camera lucida in clove oil under a cover glass, which enables one to turn the older limb buds to any position desired. As only the gross morphology of the melanophores was to be studied, no staining methods were necessary.

## THE BEHAVIOUR OF THE ADEPIDERMAL MELANOPHORES IN THE OUTGROWING LIMB BUD

The hind limb area lies lateral to the anal folds, and its skin is underlain by a network of adepidermal melanophores with rather small meshes. These meshes may measure occasionally only 30–50  $\mu$  in diameter, while those of the belly region or the flanks of the body are much larger and measure 150  $\mu$  or up to 300  $\mu$ . In individual larvae much variation in the size of the meshes occur, but those surrounding the anus are always much smaller than those cephalic or lateral to it. The area of the limb region lies in a transitory region, the meshes proximal to the anus being always smaller than those on the lateral side (Figure 1).

In the earliest stage observed, the limb bud forms a flat cushionlike mesenchymal disc of about 0.15 mm diam. (Figure 1). In this stage already, the adepidermal net is severed in several places where the bud presses against the overlying skin. Degenerating cell branches can be seen and the free end of several melanophore filaments can still be seen pointing towards each other.

In a slightly older stage of 0.25 mm diam. (Figure 2) the mesenchymal limb bud is already globular. The melanophore network is largely interrupted; some areas are completely free; degenerating cell branches are still visible, and in some places they indicate the direction of former connections. The melanophore on the lower right sends out a regenerating filament.

In a further stage 0.30 mm diam. — 0.40 mm length (Figure 3) the basal meshes are drawn over the limb, while the apical meshes are severed and find their continuation on the lower side of the bud (not drawn). The apical part of the outgrowing bud remains clear of melanophores.

In a limb bud of 0.35 diam. and 0.65 mm length (Figure 4), the picture becomes more complicated. The meshes surrounding the base of the limb have sent out a few regenerating branches, trying to reestablish the old network. In the basal third, a few cells have migrated and have already formed a new net consisting of a few meshes which are much smaller than those of the original net. A few unbranched cell strands which have apparently not divided, migrate towards the middle of the bud. On the left side a group of typical branched migrating cells have emerged from the network above, and start migrating towards the apical region.

The same components can also be seen in a slightly longer limb bud of 0.32 mm diam. and 0.95 mm length (Figure 6). At the base we find an abortive net into which branched cells which have divided also this time, are included. Once more elongate partially unbranched melanophore strands penetrate on both sides towards the limb apex. In the middle region "migrating forms" have established a new net and isolated mesh is found in the apical part.

The "migrating type" of adepidermal melanophores is shown in Figures 5, 7 and at higher magnification. The cells are triangular or elongated, sending out many branched filaments. The youngest cells may be isolated, but soon their branches adhere to each other and to those of other cells and in this way they start forming a new regenerating network. In the upper part of Figure 7 a few meshes of an older reestablished net can be seen and at the right corner a diagonally situated branch which apparently has been drawn away (or migrated) from the original net. The space between them is filled in by a number of "migrating forms", which in the centre have already established a new net, while the peripheral parts still show many free branches.

In comparing the type of "migrating" adepidermal melanophores in the outgrowing limb bud with those occurring in the regenerating tail (Bytinski-Salz and Elias 1956, Figures 31-34) the following differences can be noticed: the cells are more flattened, the cell body and its branches broader, the nuclei more distinct and the pigment granules more scattered. The edges of the cells are sometimes drawn out into a thin fan shaped membrane from which the longer filaments may branch off. The explanation may perhaps be that in the limb bud the rather compact mesenchymal core presses the melanophores against the elastic border lamella and thereby flattens their bodies, while in the tail they are imbedded with their underside contiguous with loose connective tissue. A similar type of flattened melanophore is also found in the melanophores of the peritoneum in hydropic *Pelobates* larvae (Bytinski-Salz 1956) where the changes of form are still more accentuated.

In a later stage of 1.3 mm length, the limb becomes spatulate and the knee joint is indicated. Figure 8 shows many migrating forms and a dense association of multibranched melanophores in the apical part. Almost no net has been reestablished in the basal part and most of the migrating cells seem to be unconnected with each other.

In a 1.6 mm long limb (Figure 10) the toes begin to differentiate. The net established in the knee and lower leg region begins to break up and its melanophore strands are seen migrating into the developing toes. In comparison with the former picture (Figure 8), the pigment cells are elongated, with fewer and shorter branches which don't show the typical "migrating forms". They give the impression that they had been drawn out from the preexisting net situated more proximally and the many branches pointing towards each other clearly indicate these earlier connections. In Figure 9 a part of the reestablished net of adepidermal melanophores of the same stage is drawn at higher magnification, showing the pigment strands at rest and still closely connected with each other.



In the upper and lower region of the leg, this picture, as drawn in Figure 10, remains more or less stable for the time being until the beginning of metamorphosis. Though the adepidermal melanophores still show a number of cell divisions (Figure 11, above), their rate seems to be inadequate to compensate for the increase of the surface of the skin covering the outgrowing limb and a complete network is not restored. But in the outgrowing toes (3.5–3.6 mm limb length) typical migrating forms are still found. In Figure 11, they are still connected with the resting proximal pigment strands, while in Figure 12, a large number of isolated migrating cells can be seen.

#### THE EPIDERMAL MELANOPHORES

In tadpoles of 20 mm body length, the back and parts of the sides are already covered densely by fully developed epidermal melanophores, while in the belly region, the lower flanks and the base of the lower tail fin as well, they are still absent, though sometimes an isolated melanophore may be observed here and there. In the skin covering the blastoma of the posterior limb bud they are absent in the majority of the cases. They first become apparent when the limb bud is about 0.8 mm long and very quickly increase their number. In the epidermis of the basal half of a bud 1.3 mm long (Figure 13), they show the typical form of young epidermal melanophores, a large cell body and only a few slender unbranched or very slightly branched filaments. They migrate distally together with the outgrowing epidermis. In a bud of 1.6 mm length, the number of filaments per cell is increased, these becoming more and more branched (Figure 9). In this way the cell acquires the form of the typical mature epidermal melanophore. Their number increases greatly and in an adult mature larva just before the beginning of metamorphosis (Figure 15), the cells may form a very dense network, the filaments attaching themselves to those of other cells or crossing each other at different levels. They surround the developing gland cells, without, however, entering their space, which remains empty. With the onset of metamorphosis they begin to degenerate.

#### THE SUBEPIDERMAL MELANOPHORES

The subepidermal melanophores begin to appear rather late during development. In tadpoles of 18–20 mm body length they begin to appear on the dorsal side and they become apparent in the hind limb when it is about 1 mm in length, though some of the pigment cells in the middle of Figure 4 (0.65 mm bud length) may already belong to this cell type. They are recognizable by their large flat aster-like cell body, and the many fine filaments contain a relatively smaller amount of pigment than the subcutaneous melanophores (Figure 8, 12). In later stages they increase their number very much, finally forming a very dense pigment sheet below the adepidermal melanophores. Figure 11 shows, in the upper part, this subepidermal melanophore sheet from above, and in the toe in an optical section. In the upper part of Figure 12, the individual cells are still very apparent. During later larval life, the subepidermal

melanophores form the main colouring component of the skin and persist during metamorphosis.

#### THE SUBCUTANEOUS MELANOPHORES

The subcutaneous melanophores are the first to appear during development and are present already at the beginning of the formation of the tail fin. At the beginning of the outgrowing limb bud, they are distributed all over the body, in the belly region however, in relatively smaller numbers. The limb area usually contains very few (1-5) pigment cells of this type and they may be imbedded within the limb mesenchyme. They multiply early and are present in distinctive numbers already in a bud of 0.65 mm length (Figure 4). In a later stage (Figure 8), they are present in large numbers on the dorsal side of the hind limb just below the skin. With the onset of tissue differentiation, part of them migrate into the spaces filled with connective tissue and surround the periosteum, the muscle fasciae, the nerve fibers and the walls of the differentiating blood vessels. The cells are always heavily pigmented, circular, flat with very broad branched lobes. In earlier stages, their branches are usually found in a state of pigment contraction to medium expansion; later the pigment of the cells expands more (Figures 9, 14). Figure 14 shows an optical section through the skin of the thigh at the beginning of metamorphosis, when the pattern of dark pigment spots just becomes apparent. It shows the sheet of subcutaneous melanophores directly below the skin and above it, strands of degenerate adepidermal melanophores and above these, the epidermal melanophores.

#### DISCUSSION

In earlier papers (Bytinski-Salz 1938, 1955; Bytinski-Salz and Elias 1938; Elias 1936, 1937, 1939, 1941, 1942) the melanophores of the Discoglossid genera *Discoglossus* and *Bombina* were studied under normal and experimental conditions. Special consideration was always devoted to the behaviour of the adepidermal melanophores because these exhibit a pronounced bilateral symmetry or even asymmetry (all other melanophores are radially symmetrical), and because their cell branches attach themselves to those of other cells, forming in this way an adepidermal net consisting of polygonal (*Discoglossus*) or rectangular (*Bombina*) meshes. As Elias (1936) and Bytinski-Salz and Elias (1938) have pointed out, these cells always preserve their individuality; their filaments stick to each other, forming multiple cell strands, but the cells never fuse to form a syncytium. Free melanophore strands cross each other at right angles at the edge of the tail fin in *Discoglossus* only, their direction being dependent on the intimate structure of the border lamellae consisting of an orthogonal system of connective tissue fibers (for *Bombina*, see Rosin 1946). This occurrence is very similar to the behaviour of the adepidermal melanophore net covering the body of *Bombina* (Elias 1936, Bytinski-Salz 1938). On the other hand, in the Zürich population of *Bombina* unpigmented adepidermal melanophores are found which form a typical polygonal network similar to that in *Discoglossus* (Bytinski-Salz



1938, Elias 1939). Thus, there is no fundamental difference between the adepidermal melanophores in both species and their behaviour seems to depend on their environment especially on the condition of the surrounding tissues.

The behaviour of the adepidermal melanophores in *Discoglossus* was studied during their development from radially symmetrical pigment cells, their changes into the melanophores of the pigment net (Elias 1937) and the behaviour of the net during growth (Elias 1941). Their regenerative and migrating potencies were tested in experiments presenting different environmental conditions:

1. within enclosures of skin cuts; the epidermis and cutis display regenerative movements, while the subcutaneous tissue remains at rest (Bytinski-Salz and Elias 1938, Bytinski-Salz 1955);
2. the cornea after extirpation of the eyeball; the old cornea remains at rest, while the influence of the eye, detrimental to the migration of melanophores into the cornea space, is removed (Bytinski-Salz 1960);
3. in the regenerating tail bud; the new tail is formed entirely out of undifferentiated regenerating tissue, while the active migration of melanophores from the stump is caused by "regenerative stimuli" (Bytinski-Salz and Elias 1938);
4. in the outgrowing limb bud; the mature skin and the network of adepidermal melanophores attached to it are "reactivated" by the outgrowing limb bud and caused to increase their area and multiply their number.

The skin readily responds to the stimuli of the outgrowing limb bud, which it always covers in normal thickness. The adepidermal melanophores respond to a lesser degree. Due to the pressure of the mesenchymal bud on the skin, branches of the melanophore net at rest degenerate. The remaining branches of the net are then passively drawn out into the outgrowing bud. The cells of the original network divide and try to form a new net, consisting, however, of smaller meshes. This restored net completely corresponds to the net found in the regenerating tail (Bytinski-Salz and Elias 1938, Figure 35). Only after a certain delay, free "migrating forms" of pigment cells grow out from the original net and migrate actively into the limb, even down to the tip of the toes. They try to reform polygonal networks by attaching their cell filaments to each other and in many cases such nets also consisting of meshes smaller than the original one, can be observed.

But these, as well as the earlier restored nets, are again broken up by the tension of the outgrowing limb. Sometimes old "resting type" melanophores are included in the network formed by young "migrating form" melanophores as also occurs in the regenerating tail (Bytinski-Salz and Elias 1938, Figure 30). But while the rate of growth of the developing limb increases up to and after metamorphosis, the mitotic rate of the adepidermal melanophores decreases and becomes nil at metamorphosis when the cells degenerate. So the melanophores cannot keep up their rate of multiplication to cover the entire limb with a continuous net as found on the remainder of the body. It may be said that they "try to do their best", but they never succeed. Outgrowing groups of "migrating type" cells close in together, and form isolated patches

of melanophore nets, but a net completely covering the whole limb is never formed. These patches of network are comparable to those partially developed nets found in regenerating tails in *Bombina* (Bytinski-Salz 1938). There, in younger larvae the regenerate adepidermal network is complete as the rate of division of the pigment cells is synchronous with the growth rate of the other tissues. In older larvae, though the rate of regeneration of the tail is also slowed down, the rate of multiplication of the adepidermal melanophores is still less, and in this way isolated patches of nets are found unconnected with the base and with each other (ibid. Figure 24).

The form of the "migrating type" pigment cells in the outgrowing limb and the regenerating tail is very similar; in both cases cells with triangular or elongate bodies and long slender branched filaments are found. In the limb bud these cells seem to be flatter and the branches to be more dendritic, but these differences, as was pointed out earlier (P. 3), seem to be due to mechanical conditions of the surrounding tissues. In both cases these cells are very similar to the young outgrowing adepidermal melanophores before or during their first stage of net formation as described by Elias (1937, Figure 20.)

Concerning the other types of melanophores, not much new can be added to what has been said already in earlier papers (Elias 1937, Bytinski-Salz and Elias 1938, Bytinski-Salz 1960). The forms found in the limb bud are the same as found in other parts of the body and their sequence of appearance in the limb is the same as on the back; they only appear later; i.e. first subcutaneous melanophores, adepidermal melanophores, epidermal melanophores and finally subepidermal melanophores. Additional evidence is given concerning their form and structure, but simple observation cannot contribute to the question of their origin.

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## PLATE I

Figure 1 Hind limb bud 0.15 mm diam. 100 x

Figure 2 Hind limb bud 0.25 mm diam. 100 x

Figure 3 Hind limb bud 0.30 mm diam. 0.40 mm long 100 x

Figure 4 Hind limb bud 0.34 mm diam. 0.65 mm long 100 x

In figures 1-3 only the adepidermal melanophores are drawn, in figure 4 all melanophores are drawn.

Figure 5 Migrating adepidermal melanophores from limb bud 1.15 mm 200 x





## PLATE II

- Figure 6 Hind limb bud 0.32 mm diam, 0.95 mm long, only adepidermal melanophores drawn 100x  
 Figure 7 Adepidermal melanophores from a bud 0.91 mm long 200 x  
 Figure 8 Hind limb spatulate 1.3 mm long, all melanophores drawn 80 x  
 Figure 9 Melanophores from a hindlimb 1.6 mm long 250 x



# PLATE III

- Figure 10 Hind limb, 1.6 mm, beginning formation of digits, only adepidermal melanophores drawn 100 x
- Figure 11 Hind leg 3.5 mm long, 2nd toe 100 x
- Figure 12 Hind leg 3.6 mm long, 2nd and 3rd toe 50 x
- Figure 13 Epidermal melanophores from limb 1.3 mm long 210 x
- Figure 14 Edge of the thigh from full grown tadpole prior to metamorphosis 210 x
- Figure 15 Epidermal melanophore net from full grown tadpole prior to metamorphosis 210 x

# THE MALE AND NYMPH OF *IXODES KAISERI* ARTHUR, 1957

DON R. ARTHUR

*Department of Zoology, University of London King's College*

## ABSTRACT

Females of *Ixodes kaiseri* Arthur 1957 were first collected from young common Egyptian foxes, *Vulpes vulpes aegyptiaca* (Sonnini 1816) at Burg El Arab, Mariut, Western Desert Governate, Egypt by Hoogstraal and his collaborators in 1955 and 1956. At that time, it was represented by only small populations of females in limited geographical areas of Egypt. More recently, Professor Theodor of Jerusalem asked me to examine collections of *Ixodes* ticks from Israel and among the specimens were females, males and nymphs of *I. kaiseri*. In this paper, the male and nymph of *I. kaiseri* are described for the first time, and additional records of other *Ixodes* from Israel are recorded. Specimens of males and nymphs of *I. kaiseri* are deposited in the Department of Parasitology, The Hebrew University of Jerusalem.

## *Ixodes kaiseri* Arthur, 1957

### Male:

Length from tips of scapulae to posterior margin of body, 2.13 mm, maximum width 1.42 mm. Body elongate oval, narrowing more strongly in front. Colour brown, legs paler.

Capitulum: (Figure 2A,B) Length from tips of palpi to posterior margin of basis capituli, 0.432 mm. Greatest width 0.271 mm at level of insertion of palpi. Basis small, posterior margin straight or only slightly concave, elevated and continued into a narrow neck. Surface convex with few punctations. Cornua absent. Palpi short, stout and club-like, tumescent dorsally with numerous spinose hairs. Length of palpal segment II, 0.12 mm, of palpal segment III, 0.11 mm. In ventral view basis long, lateral margins nearly straight and diverging to base of palpi, a broad inverted V-shaped groove in the position as indicated by the figure. Auriculae lacking, but slight swellings where they do arise in other ticks. Hypostome shorter than palpi, broad, notched apically; shape and dentition as indicated by the figure. Length of toothed portion about 0.1 mm (Figure 2c).

Scutum: Surface smooth, shining. Shape as in Figure 1A. Punctations of moderate size and number, predominantly along the periphery. Emargination well defined, scapulae short and pointed. Pseudoscutum faintly indicated. Cervical grooves divergent, represented by two short, deep depressions anteriorly; three longitudinal depressions in the posterior half, lateral carinae absent. Hairs short and scattered, more numerous posteriorly and on the prominent marginal body fold.



Ventral plates: Pregenital plate indicated by an irregular sclerotization. Median plate about as long as anal plate. Adanal plates equally broad anteriorly and posteriorly. Short fine hairs on all plates, being, most numerous on epimerals. Punctuation

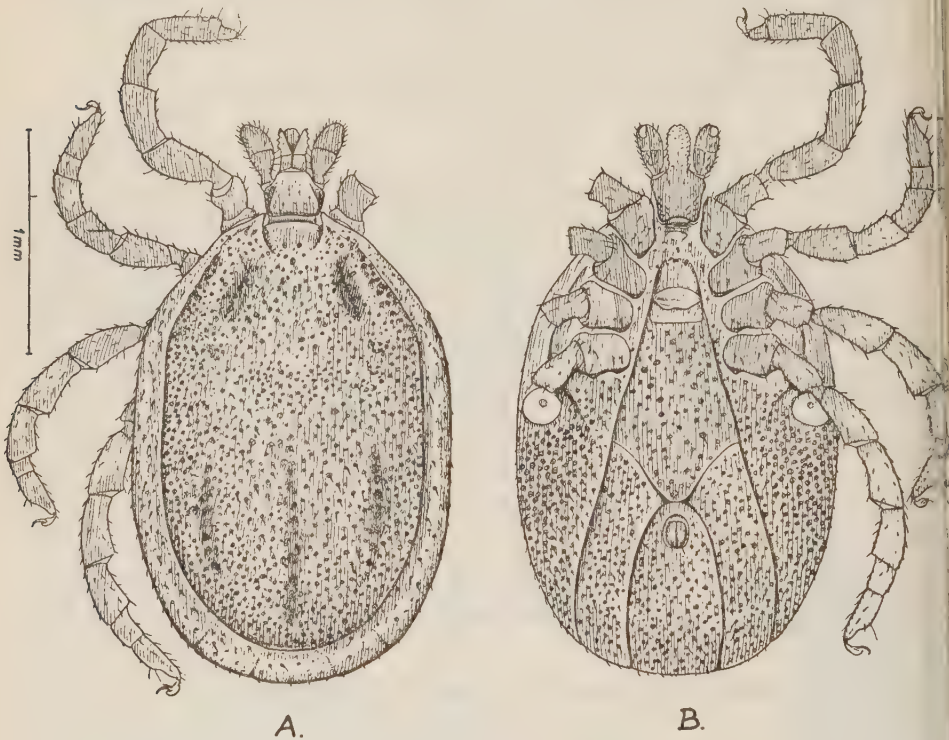


Figure 1  
*I. kaiseri* male. A. Male — dorsal; B. Male — ventral.

moderate in size and number, shallow. Genital and anal grooves well defined. Anal grooves either ogival or broadly rounded in front of anus (Figure 1B), very slightly convergent posteriorly (There is some variation in this character for the front of the anal groove may be straight).

Legs: Moderate in length and size. All coxae lacking external spurs, but occasionally saliences on coxae III and IV; short, broad tapered internal spur on coxa I, weak internal saliences on II-IV. Trochantal spurs lacking (Figure 2E). Tarsus I with steep hump, progressively less steep from tarsi II to IV. Length of tarsus I, 0.42 mm, metatarsus I, 0.35 mm. Length of tarsus IV, 0.42 mm, metatarsus IV, 0.38 mm. Hairs of moderate length and strong (Figure 2D).

Spiracular plate: Subcircular, greatest dimension about 0.27 mm. Goblets moderate in number and size (Figure 2F).

LYMPH:

Capitulum: Length 0.37mm, width of basis 0.23 mm. Basis much as in female; cornua well developed, pointed and widely separated; posterior margin straight or slightly irregular. Palpi similar to those of female, length of palpal segment II, 0.13 mm, palpal segment III, 0.09 mm (Figure 4A). Ventrally, basis broadly rounded posteriorly, lateral margins divergent to palpal insertion. Auricular ridges present posterior

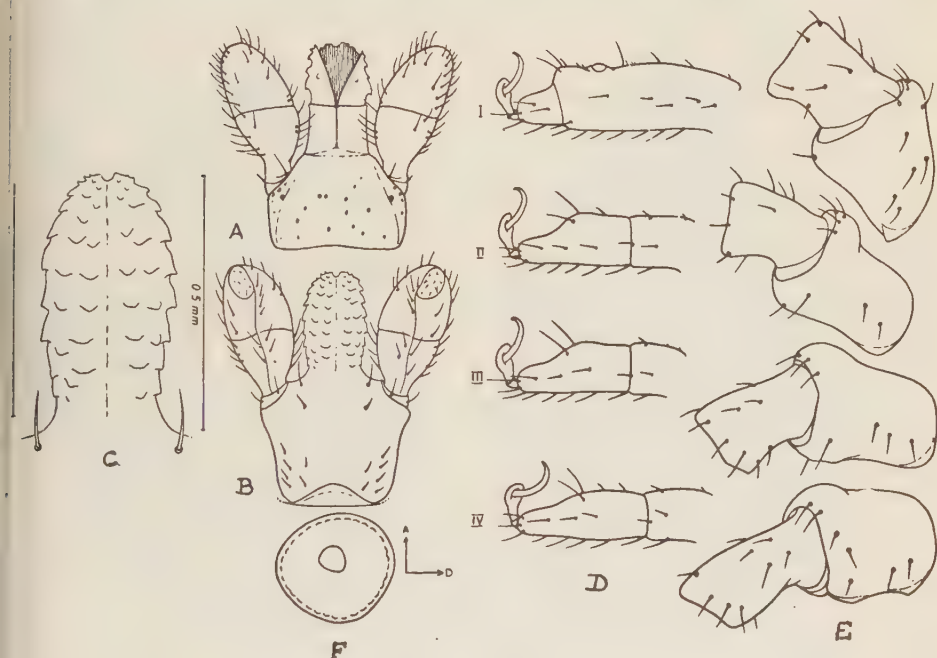


Figure 2.

*kaiiseri* male. A. Capitulum — dorsal; B. Capitulum — ventral; C. Hypostome; D. Tarsi I-IV; E. Coxae and trochanters I-IV; F. Spiracular plate — A = anterior, D = dorsal.

which the basis is very slightly constricted (Figure 4B). Hypostome slightly longer than palpi; dentition behind corona as 6 rows of  $2\frac{1}{2}$  files. Length of toothed portion 0.08 mm (Figure 4C).

Cutum: Length 0.43–0.45 mm, width 0.38–0.40 mm. Shape as in figure 3A. Scapulae short and sharply pointed. Lateral carinae absent. Cervical grooves convergent at first and then divergent to postero-lateral margin. Hairs few, short, scattered.

Eggs: External spurs on coxae I-IV, postero-internal edge of coxa I as a marginal prominence (Figure 4E).

Spiracular plate: Transversely oval. Goblets fewer and relatively larger than in female.

RELATED SPECIES AND REMARKS

Separation of *I. hexagonus* Leach from *I. kaiseri* has been discussed elsewhere (Arthur 1957) but problems are also likely to arise in separating this species from *I. canisuga* Johnston and *I. passericola* Schulze. Separation is, however, possible by use of the following dichotomous keys.

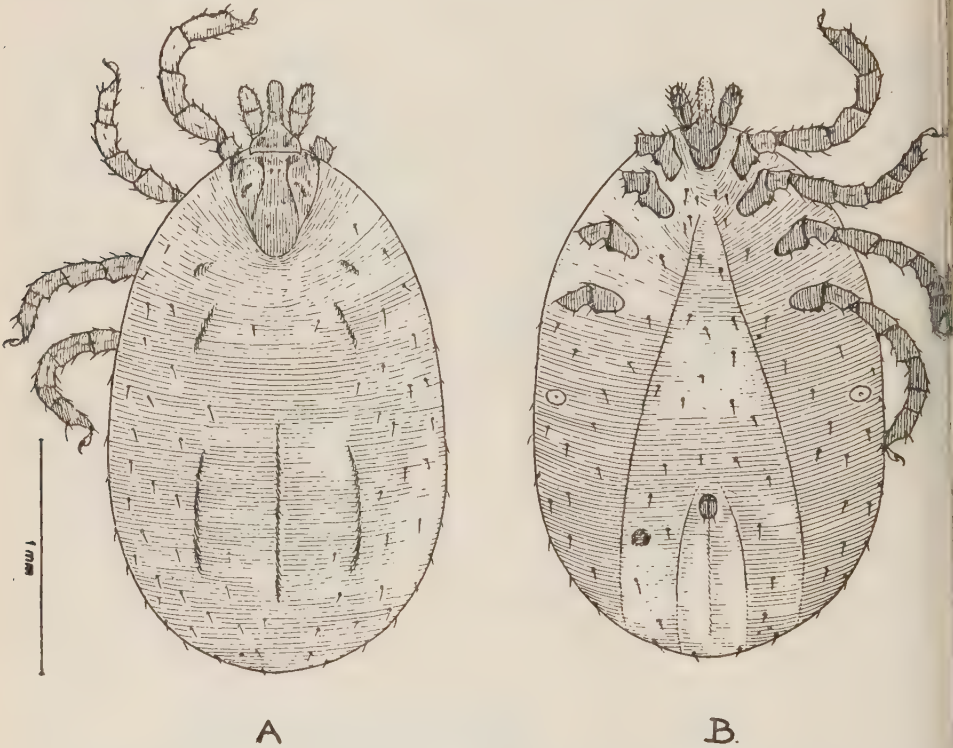


Figure 21.

*I. kaiseri* nymph. A. Fully fed nymph — dorsal; B. Fully fed nymph — ventral.

KEY FOR SPECIES OF *Ioxdes*

Females:

1. Auricular ridges absent . . . . . *I. kaiseri*
- Auricular ridges present . . . . . 2
2. Palpi very broad and relatively short; scutum tapering strongly behind mid-length; larger species . . . . . *I. canisuga*
- Palpi less broad, scutum less strongly tapered behind mid-length; smaller species . . . . . *I. passericola*

Males:

1. Basis capituli 1.7 times broader than long (as in Figure 203 of *I. canisuga*, Nuttall *et al.*, 1911). Palpi short and very broad,



(length/breadth ratio 1.8–2.0:1.0) . . . . . *I. canisuga*  
 Basis capituli 1.5 times broader than long (as in Figure 2A);  
 Palpi longer and less broad (length/breadth ratio 2.4–2.5:1.0) . . . *I. kaiseri*  
 males of *I. passericola* require re-investigation as previous descriptions are based  
 mounted preparations.

nymphs:  
 Coxae I–IV lacking external spurs . . . . . *I. passericola*  
 Coxae I–IV with slight protuberances at postero-lateral angles . . . 2  
 Cornua lacking . . . . . *I. canisuga*  
 Cornua present as pointed projections, widely separated . . . *I. kaiseri*

# HOSTS AND DISTRIBUTION OF *I. Kaiseri*

*Ursus vulpes aegyptiaca*, 2 females; *Hyaena hyaena*, 2 females, 2 nymphs, larvae?;

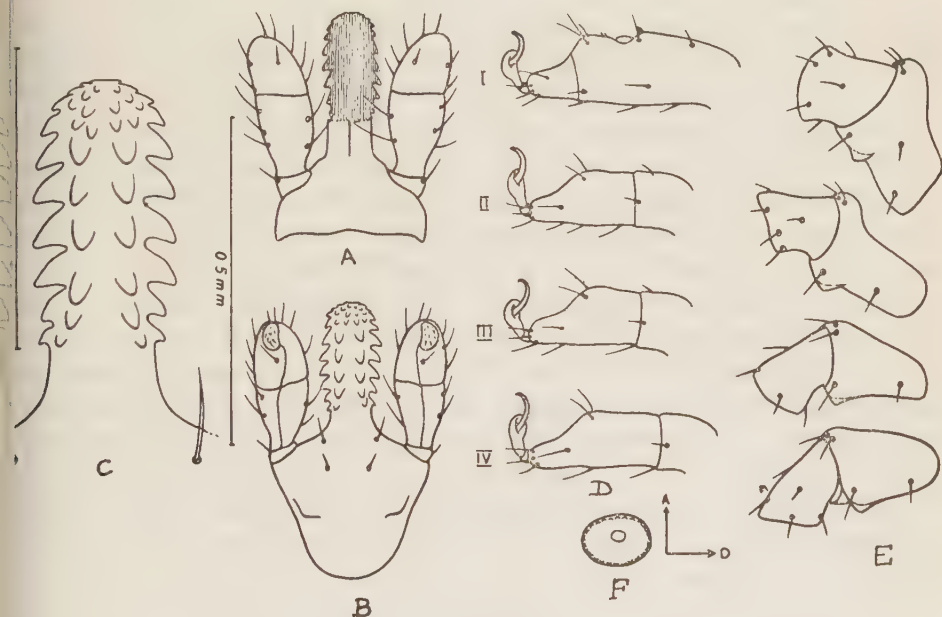


Figure 4.

*I. kaiseri* nymph. A. Capitulum — dorsal; B. Capitulum — ventral; C. Hypostome; D. Tarsi I–IV;  
 E. Coxae and trochanters I–IV; F. Spiracular plate — A = anterior, D = dorsal.

*Meles meles*, 2 females, 2 males, 2 nymphs; *Erinaceus europaeus*, 2 females, 2 males,  
 nymphs; *Hystrix indica*, 1 female, 1 nymph; *Felis chaus*, 1 female, 1 nymph;  
*Ursus*?, 1 female. Nymphs, males and females and possibly larvae occur together on  
 similar hosts. Apart from the Egyptian record, we now have the following from  
 Israel: *Hyaena hyaena*, Ein Harod, II. 1940, 3 females; *Hyaena hyaena*, Bat Shlomo,  
 X.57; *Meles meles*, Hartuv, 25.III.54, 2 nymphs; *Meles meles*, Ein Harod, 5

females, 1 male; *Meles meles*, Jerusalem, 4.V.53, 1 female; *Meles meles*, Bab el Wa 12.I.54, 2 nymphs; *Meles meles*, Mishmar Ha'emek, 8.II.55, 1 male, 2 females, nymphs (1 nymph in the present paper); *Meles meles*, Tivon, 17.I.56, 3 females; *Meles meles*, Tivon, 17.I.56, 2 females, 1 nymph; *Erinaceus europaeus*, Jerusalem, VII.36, 3 females, 3 males (1 male is figured in the present paper); *Erinaceus europaeus*, Yesod Hamaalah, VIII.28, 1 nymph; *Histrix indica*, Mishmar Ha'emek, 17.II.55, 1 female, 1 nymph; *Felis chaus*, Mishmar Ha'emek, 9.II.57, 1 female, 3 nymphs; *Felis? ocreata*, Mishmar Ha'emek, XII. 58, 1 female; *Vulpes vulpes*, Yassur, 15.I.57, 1 nymph, 5 larvae.

#### OTHER *Ixodes* RECORDED FROM ISRAEL

*Ixodes passericola* Schulze: 2 females, 2 males, 1 nymph nest of *Petronia petronia* Jerusalem, 1.VI.54; (4 females) *Petronia petronia*, Jerusalem, 5.VI.56.

*Ixodes vespertilionis* Koch, 1844: 1 nymph, 2 larvae, *Myotis capaccinii*, Rosh Pina, 20.IX.46; 1 female, *M. myotis*, Kfar Kana, 25.VI.27; 1 nymph, 4 larvae, *M. myotis*, Palestine (no exact data), 23.VIII.46; 1 nymph, *M. myotis* Khasas, 7.IV.47; 1 nymph, *Miniopterus schreibersi*, Palestine, 29.IX.46. *Ixodes simplex simplex* Neumann 1900: 1 female, *Miniopterus schreibersi*, Huti, 28.VIII.47; 1 female, *Miniopterus schreibersi*, Usbo, 30.V.47; 1 female, *M. schreibersi*, Rosh Pina, 21.IX.46; 1 female, 1 nymph, *M. schreibersi*, Usbo, 30.V.27; 2 nymphs, *M. schreibersi*, Usbo, 30.V.47; 1 larva, *M. schreibersi*, Herzlia, 5.I.47; 1 nymph, *M. schreibersi*, Palestine, 29.IX.46; 1 nymph, *M. schreibersi*, Dellata, 25.X.46; 1 nymph, *M. schreibersi*.

*Ixodes redikorzevi theodori* Warburton, 1927: 1 female, human, Nahalal, no further data; 1 female, *Spalax ehrenbergi*, Kfar Ivri, XII. 42; 1 female, *Meriones tristrami*, Mishmar Ha'emek, 7.XII.52; 1 female, Human, Hartuv, 27.XII.52; 1 female, *Asinus flammeus*, Ma'abarot, 20.II.53; 1 female, Human, Hadera, II.55; 1 female, Human, Petah Tikva, 20.IV.54; 1 female, child's head, Hartuv, 9.II.56; 2 females, *Rallia aquaticus*, Hefziba, 14.I.56; 4 males, in burrow of *Microtus guentheri*, Mishmar Ha'emek, 4.XII.55; 1 female, *Ovis tarda*, Evron, 7.I.57; 3 females, 1 male, *Charadrius apricarius*, Shave Zion, 3.XII.57.

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I am grateful to H. Hoogstraal, NAMRU 3, Cairo, Egypt, for arranging for the drawings of the male and nymph of *Ixodes kaiseri*. It has also been my privilege to discuss the distribution of *I. kaiseri* in Israel with Professor Theodor.

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# CRITICAL LIST OF THE *HISTERIDAE* (COL.) FROM ERETZ ISRAEL

GIUSEPPE MÜLLER

*Museo Civico di Storia Naturale, Trieste*

## ABSTRACT

This paper presents a critical and annotated list of 55 species of *Histeridae*, of which 23 are new for the fauna of Eretz Israel, together with a description of two new subspecies: *Saprinus prasinus* Er. ssp. *aeneomicans* ssp. nov. and *Hister uncinatus* Ill. ssp. *reductus* ssp. nov.

## INTRODUCTION

At the request of my colleague, Dr. H. Bytinski-Salz, Chief Entomologist of the Division of Plant Protection at the Ministry of Agriculture, Tel Aviv, I have compiled a up-to-date critical catalogue of the *Histeridae* known from Eretz Israel. Most of the older information in this connection is contained in two faunistic catalogues prepared by John Sahlberg: "Coleoptera Levantina, mensibus Februario et Martio 1906 in Palaestina et Aegypto inferiore collecta," *Oefver. Finska Vetensk. Societ. Forhandl.*, **45**, 1902-03 (*Histeridae* det. Reitter," pp. 19-20) and "Coleoptera Medioranea Orientalia," *ibid*, **50**, 1913, pp. 85-89. Additional descriptions and mention of localities regarding the region are to be found in the monographs of Marseul (*Monographie des Histerides*," *Ann. Soc. Ent. France, Ser. III*, **1-8**; *Ser. IV*, **1, 2**, 1853-1862); Schmidt ("Best. Tab. Europ. Coleop., **14: Histeridae**," 1885); Reichhardt ("Beiträge zu einer Monographie der *Saprininae*. I.", *Mitt. Zool. Mus. Berlin*, **18**, 1932; "Faune de l'URSS" *Coleoptera*, V. 3: *Histeridae*, **1**," 1941); as well as in various briefer papers by Bickhardt, Müller, Reichardt, Reitter, etc., as mentioned in the text. Most of the *Histeridae* known from Eretz Israel up to the preparation of the present publication have been included in Bodenheimer's "Prodromus Faunae Palaestinae," (*Mem. Inst. Egypte*, **33**, 1937, p. 121). The species not listed in this catalogue are marked with an asterisk (\*) in the present paper.

In addition, Dr. H. Bytinski-Salz, during his numerous collecting trips throughout Israel, has gathered together a large number of *Coleoptera*, and has submitted the *Histeridae* to me for diagnosis and revision. As a result, the number of species established for Israel rose from 32 to 55, including 2 new subspecies; besides these, three other species, the occurrence of which has not yet been established with certainty, are in need of revision. Specimens which have been submitted to me for identification by Dr. Bytinski-Salz are marked with an exclamation point (!).



As in all my faunistic work, I have also cited in the present catalogue the literature used for the diagnosis of each species. The species thus becomes diagnostically fixed for reference to a species by means of the author's name alone is insufficient, particularly in the case of old descriptions, which are often applied to species only recently defined with precision. Citations of the literature for each species are followed by the habitats, which are described as accurately as possible. This method, in my opinion, is more practical than the division of the species, as by Bodenheimer, into Iranian, Turanian, Mediterranean, Holarctic, Saharo-Sindian and Euro-Siberian faunal elements; the nomenclature of the biogeographical regions, subregions, districts, etc. is not yet uniform and the zoogeographical names adopted by individual authors are therefore, ambiguous. The habitats are followed by the place of collection, with exact sources and remarks on variability, synonymy, systematic position, etc.

### Gen. *Teretrius* Erichson

#### \* *T. acaciae* Reitt.

Reitter, *Deutsche Ent. Zeitschr.*, 1900, p. 83; Müller, *Ent. Blatt.*, 1937, p. 99.

Described from S. Tunisia; collected from dead twigs of *Acacia tortilis*, in the tunnels of *Xylopertha forficula* Fairm. Eretz Israel: Wadi Fukra, 3. VII. 42, one specimen (By.-S!).

### Gen. *Onthophilus* Leach

#### \* *O. striatus* Forst (1771)

Marseul, *Monogr.* 1856, p. 560; Schmidt, *Best. Tab.* 1885, p. 321; Ganglbauer, *Käf. Mitteleur.* 1899, p. 401; Reichardt *Faune URSS* 1941, p. 354.

Europe, Asia Minor, Cyprus, Syria.

Eretz Israel: Canae, 29. III. 04 (Sahlberg 1913).

#### \* *O. affinis* Redtb. (1849)

Marseul, *Monogr.* 1856, p. 560; Schmidt, *Best. Tab.*, 1885, p. 321; Ganglbauer, *Käf. Mitteleur.*, 1899, p. 401; Reichardt, *Faune URSS* 1941, p. 355.

Pontic species, from Asia Minor and Syria to Italy; northwest-wards to Austria and Bohemia.

Eretz Israel: Bethlehem, 22. II. 96 (Sahlberg 1902-03).

#### *O. bickhardti* Reitt.

Reitter *Ent. Blätt.* 1909, p. 180; Reichardt *Faune URSS* 1941, p. 355.

Described from one specimen from Jerusalem. Bickhardt (in Wystman, *Genera Insectorum Histeridae* 1917, p. 65) mentions only Asia Minor, but not Eretz Israel. Reichardt 1941, p. 90, who knew this species only from Reitter's description, gives Syria as patria.

*W. sulcatus* Fabr., (1792)

Marseul *Monogr.* 1856, p. 554; Ganglbauer *Käf. Mitteleur.* 1899, p. 402; Reitter *Fauna Germ.* II 1909, p. 296; Reichardt, *Faune URSS* 1941, pp. 90 and 355. *globulosus* Schmidt *Best. Tab.* 1885, p. 321 (*nec* Olivier).

W. and S. Europe, Algeria, Asia Minor, Caucasus; sporadically also in Austria and Germany.

Eretz Israel: Bethlehem, 22. II. 96 (incorrectly cited by Sahlberg 1902-03, as *globulosus* Ol., which is found only in the W. Mediterranean basin); Jerusalem, 19. II-5. III, and Lake of Galilee, 28. III (var. *caucasicus* Reitt., according to Sahlberg, 1913, whilst Bodenheimer 1937 cites the Greek variety *cicatricosus* Reitt.). A few specimens collected by Dr. Bytinski Salz near Jerusalem (26. III. 31!) doubtlessly belong to *caucasicus* (the pronotum relatively finely dotted, with two fine paramedial sharply separated, parallel keels in front of the basis).

#### Gen. *Plegaderus* Erichson

*P. otti* Mars.

Marseul, *Monogr.*, 1856, p. 271; Schmidt, *Best. Tab.*, 1885, p. 320; Ganglbauer, *Käf. Mitteleur.*, 1899, p. 399; Reichardt, *Faune URSS.*, 1941, p. 359.

Mediterranean countries.

Israel: Carmel, 10.I and Ginegar, 3. III, under the bark of *Pinus halepensis*; also near Asluj, 8. VII (leg. By.-S!).

#### Gen. *Abraeus* Leach

*A. convexus* Reitt.

Reitter, *Wien. Ent. Zeitg.*, 1884, p. 8; Schmidt, *Best. Tab.*, 1885, p. 323; Reichardt, *Faune URSS.* 1941, p. 364.

Described from Greece (Attica) and Israel (Haifa); according to Schmidt, also Turkey; in my collection is one specimen from Macedonia (Saloniki); according to Reichardt, lives in cattle dung.

Israel: Haifa (Reitter, 1.c.). Deir Aban, W. Judean Mts.; Jaffa, Canae, 12.II-29.III. (Sahlberg 1913).

#### Gen. *Acritus* Leconte

*A. nigricornis* Hoffm. (1803)

Müller *Verh. zool. bot. Ges. Wien* 1900, p. 301 (*Üseminulum* Küst. ♀); Gerhardt *Deutsche Ent. Zeitschr.* 1903, p. 239; Reitter, *Fauna Germ.* II, 1909, p. 297; *nigricornis* + *minulum* apud Schmidt, *Best. Tab.* 1885, p. 325 and Ganglbauer *Käf. Mitteleur.* 1899, p. 407.

Europe and Mediterranean, eastwards to Turkestan.

Israel: Jaffa, 18.II.04, in dung; and Nazareth, 29.III.04 (Sahlberg 1913).

Gen. *Saprinus* Erichson (s. lato)Subgenus *Phaonius* Reichardt*S. pharao* Marseul.

Marseul *Monogr.*, 1855, p. 399; Schmidt, *Best. Tab.*, 1885, p. 304; Reichardt *Faune URSS.*, 1941, p. 188 and 387.

Egypt (*terra typica*), Greece, Crimea, Caucasus, Syria, eastwards to Mongolia.

Israel: Givat Brenner, 8.II and Rehovot (By.-S, 2 specimens!).

Subgenus *Saprinus* (s. str.)*S. maculatus* Rossi (1790)

Marseul, *Monogr.*, 1855, p. 355; Schmidt *Best. Tab.*, 1885, p. 303; Ganglbauer *Käf. Mitteleur.*, 1899, p. 383; Reichardt, *Faune URSS* 1941, p. 375.

S. Europe, Caucasus, Asia Minor, Turkmenia, Turkestan.

Israel: Herzlia, 7.V and Urim, 12.IV (By.-S!).

Bodenheimer, in *Prodromus Faunae Palaestinae*, (1937) mentions also the following species of *Saprinus*, which were not checked by me:

*S. moyses* Marseul.

The occurrence of this species in Israel is very probable. General distribution: Canaries, Africa, Syria, Iran (after Reichardt, *Faune URSS* 1941, p. 384).

*S. aeneus* Fabr.

Although it is widely distributed in Europe and Siberia, the occurrence of this species in Israel is less certain, as the exact differentiation of related species from Asia often encounters difficulties.

*S. ornatus* Erichson (1834)

Marseul *Monogr.*, 1855, p. 360 and 1862, p. 439; Schmidt, *Best. Tab.*, 1885, p. 304; Müller, *Ann. Mus. Genova LVI*. 1933, p. 187; Reichardt *Faune URSS.*, 1941, p. 190 and 375; *osiris* Marseul *Monogr.* 1862, p. 440.

N. Africa, Asia Minor, Turkestan, Mongolia.

Israel: Ra'anana, 25.III; Mishmar Hanegev, 2.III; Beersheba, 14.III; Gvulot, 30.V; 'Ein Feshra, 6.IV (By.-S!, few specimens!).

\* *S. semipunctatus* Fabr. (1798)

Marseul *Monogr.*, 1855, p. 377; Schmidt *Best. Tab.* 1885, p. 304; Ganglbauer *Käf. Mitteleur.*, 1899, p. 383; Reitter *Fauna Germ.* 1909, p. 29; Reichardt *Faune URSS.*, 1941, p. 206 and 377.

Mediterranean, S. Russian steppes, Caucasus, Turkmenia, Turkestan. Southward to Cap Verde islands, Tripolitania and Egypt; in S. Arabia and Eritrea already represented by the closely related *S. elegans*.

Israel: Ra'anana, 14.IV; Herzliya, 7.V; Rehovot, frequently; two specimens also Beersheba, 1.IV (By.-S!).



*niger* Motsch. (1849)

Marseul *Monogr.*, 1862, p. 450; Schmidt *Best. Tab.*, 1885, p. 306; Reichardt *Faune URSS.*, 1941, p. 198 and 376.

According to Reichardt, in the W. Mediterranean, Rumania, Asia Minor, Transcucasia, Turkmenia and Turkestan; I know this species also from N. Africa: Algeria (Biskra!) and Libya (Bengasi!).

Israel (Negev): Revivim, 12.V; and Urim, 28.V. (By.-S, 7 specimens!).

*semistriatus* Scriba (1790), s. str.

Müller *Ent. Blätt.*, 1937, p. 105; Binaghi and Moro, *Boll. Soc. Ent. Ital.*, 1946, p. 60. *mistriatus* partim, Ganglbauer *Käf. Mitteleur.* 1899, p. 384; Reitter, *Fauna Germ.*, 1909, p. 292; Reichardt, *Faune URSS.*, 1941, p. 220 and 379; *nitidulus* partim Marseul, *Monogr.*, 1855, p. 402; Schmidt, *Best. Tab.*, 1885, p. 306.

According to the older authors, widely distributed in the Palearctic region, but mostly confused with *S. punctatostratus* and other related species. The distribution of the true *semistriatus* has still to be defined with precision. In my experience, it is a rather southern species, frequent in Mediterranean countries, which is replaced in colder regions with a continental climate by *S. punctatostratus* Mars.

Israel: Ra'anana, 25.III and 14.IV; Givat Brenner, 8.XII; Ramat Gan, 6.IV and 14.IV (By.-S!). I also possess specimens from Jericho (ex coll. Hauser!).

*concinus* Motsch. (1849)

Marseul *Monogr.*, 1862, p. 453, Table XI, Figure 14 (nec 1855, p. 400, Figure 39), Schmidt, *Best. Tab.*, 1885, p. 306; *lateralis* ab. *vermiculatus*, Reichardt *Ent. Mitt.*, 1923, p. 239 and 242.

Greece, Caucasus, Turkey; the occurrence in Italy (after Schmidt, l.c.) appears to me very doubtful. In my collection are specimens from the Greek islands of Syros (leg. Schatzmayr) and Crete (leg. Paganetti); also from Asia Minor and Persia (Asterabad).

Israel: Beersheba, 1.IV, also Ra'anana and Rishon le Zion, 8.II, single specimens (By.-S!). The *Saprinus lateralis* from Bodenheimer's list (1937) also apparently refers to this species with rugose punctuation on the elytrae.

*chalcites* Illiger (1807)

Müller *Mem. Soc. Ent. Ital.*, 1931, p. 96; Reichardt, *Faune URSS.*, 1941, p. 237 and 382; *chalcites* auct. (partim).

Mediterranean, eastwards to Central Asia; also in tropical Africa, Arabia and India (Madras).

In Israel frequently: Carmel-Khreibe, 20.VI; Ramat Gan, 24.III., 3.V; Bat Yaim, 14.III.; Rehovot, 14.IV-15.IX; Givat Brenner, 8.XII; Ekron, 31.VII; Revivim, 12.V; (By.-S!).

\* *S. angoranus* Bickhardt

Bickhardt *Ent. Blätt.*, 1910, p. 110; Müller *Mem. Soc. Ent. Ital.*, 1931, p. 96; Reichardt, *Faune URSS*, 1941, p. 234 and 382; *chalcites* auct. (partim); also Bickhardt *Arch. Naturg.* 1921, p. 122.

Distribution (after Reichardt): Anatolia, Syria, Transcaucasia, Turkmenia, Turkestan. According to material available to me, this species also occurs on Crete and Naxos, in the Vardar valley near Salonica and on Mount Athos, as well as in Dalmatia (Castella, Spalato, Herzegovina).

Israel: Nir Am, IX.48, one specimen (By.-S!).

Remark: In the Mediterranean, in addition to *S. chalcites* and *angoranus*, there occurs a third, very similar and therefore often misidentified species, named *S. georgicus* Marseul. Only Reichardt (1941, p. 382 and Figure 122, p. 234) correctly recognized and appropriately stressed the specific independence of *S. georgicus*. *S. georgicus* has a wider distribution than *angoranus* and has been recorded from Morocco to Turkestan, and may thus occur in Israel.

\* *S. strigil* Marseul

Marseul *Monogr.*, 1855, p. 444, Table 16, Figure 70 (type: Abyssinia); Bickhardt *Arch. Naturg.*, 1921, p. 120; Müller, *Boll. Soc. Ent. Ital.*, 1938, p. 167.

This species, mentioned by Bickhardt (in Wytzman 1917) only for Abyssinia, has a much greater distribution. I possess specimens from various localities in Eritrea, Abyssinia, S.W. Africa and Senegal; according to Burgeon (1913) it also occurs in the Belgian Congo. In the E. Mediterranean, it was also found in Syria and Cyprus. In the Hauser collection I saw specimens from Gr. Balchan-Djebel in Transcaspienia.

Israel: Rehovot, 14.IV; Ramat Gan, 24.III.45 (leg. By.-S. 4 specimens!).

*S. prasinus* Erichson (1834)

Marseul *Monogr.*, 1855, p. 414; Schmidt, *Best. Tab.*, 1885, p. 307; Reichardt *Faune URSS.*, 1941, p. 244 and 385.

According to Reichardt (*l.c.*) distributed from Italy to Asia Minor and Armenia. Material in my possession from E. Mediterranean only: Greece (Reitter), Macedonia, Salonica (Schatzmayer), Rhodes (Wohlberedt), Crete (Paganetti) and Cyprus, (By.-S.).

In Israel rather frequent. In Tel Aviv III.43 and Rehovot III.48 there were collected in addition to the green type some specimens with a rather bronze coloration. Near Ra'anana (!) Dr. Bytinski-Salz found only the bronze-coloured variety, which thus appears to occur there as a distinct race: ssp. *aeneomicans* ssp. nov. Type and paratypes: Ra'anana, 25.III.48!.

*S. subvirescens* Men. (1832)

Reichardt *Faune URSS.*, 1941, p. 240 and 383; *foveisternus* Schmidt *Wien. Ent. Zeitg.*, 1884, p. 9 and *Best. Tab.*, 1885, p. 308.

Near East, Caucasus, Lower Volga, Turkmenia, Turkestan (Reichardt, *l.c.*); Mesopotamia, Assur (Pietschman!).

Eretz Israel: Jordan near Jericho, 24.V.27, one specimen (leg. O. Theodor!). Determined incorrectly as *aeratus* by Desbordes and cited by Bodenheimer (*Prodromus faunae Palaestinae* 1937, p. 121) under this name(§). Also from Ramat Gan 6.IV.1945 (Lytinski-Salz !).

A second specimen from Jericho, also determined as *aeratus* by Desbordes, is no *Saprinus* at all but a *Hypocacculus*, which cannot be determined precisely owing to its poor condition (possibly *palaestinensis* or *baudii*?).

*S. politus* Brahm. (1790)

Reitter, *Fauna Germ.*, 1909, p. 293; Müller, *Ent. Blätt.*, 1937, p. 109; Reichardt *Faune URSS.*, 1941, p. 246 and 385; *speculifer* Latreille, 1807: Marseul *Monogr.*, 1855, p. 411; Schmidt, *Best. Tab.*, 1885, p. 308; *pulcherrimus* Weber, (1801): Ganglbauer, *Käf. Mitteleur.*, 1899, p. 387.

Mediterranean, after Reichardt (*l.c.*), also in Central Europe; however, I have not yet seen a specimen from Central Europe.

Israel: frequently; Tel Aviv, 1945; Ra'anana, 25.III.45; Ramat Gan, 24.III.45; Ashdod, 11.III.42; and Rehovot, 11.III, 14.IV, 15.XI.48. Individual specimens from Bat Yam 24.III.44 and Givat Brenner 24.II.47 (all. By.-S.!).

*S. figuratus* Marseul.

Marseul *Monogr.*, 1855, p. 409; Schmidt *Best. Tab.*, 1885, p. 307; Reichardt, *Faune URSS.*, 1941, p. 245 and 384.

Mediterranean, questionably also in Near East (Reichardt, *l.c.*).

Eretz Israel: Jericho, 28.II and 11.III.96 (Sahlberg 1902-03); single specimens from Revivim, 12.V! and Ramat Gan 6.IV (By.-S.!).

*S. tenuistrius* Marseul 1855 (*sparsutus* Solsky 1876)

Eretz-Israel: according to Sahlberg (1913) near Deir Aban, W. Judean mountains, excreta of cats, 29.II.04. So far I have not seen any Israel specimens and cannot say anything as to their racial position. It should be noted that the *sparsutus* Solsky distributed in S. E. Europe and Asia is not exactly identical with the genuine *tenuistrius* Marseul from Egypt and the Daalac Islands in the Red Sea (vide my "Histeridenstudien" in *Ent. Blätt.*, 1937 p. 108).



*S. ruber* (Mars.) sensu Müller.

Müller *Ent. Blätter*, 1937, p. 110, and Reichardt, *Faune URSS*, 1941, pp. 38 and 388; Bodenheimer (1937) as *var. pilimargo* Reitt.

The genuine *ruber* Mars. from Tripolitania, Tunisia and Algeria, with red-brown elytrae, is represented in Israel by the uniformly black race *Gemmingeri* Mars. (local class: Jaffa), a specimen of which was also found at Beersheba, 7.VI (By.-S.). The elytrae are extremely finely and not densely punctate in their posterior part only. The same black race also occurs in Egypt and was described by Reitter (*Bull. Soc. Ent. Egypte*, 1915, p. 137) as *S. pilimargo*.

*S. ruber gemmingeri* differs from similarly coloured *S. gilvicornis* Erichs. in the labrum, which is rounded in front and not excised, and by the finer punctation on the front, and the pygidium, the much flatter postocular grooves on the pronotum and the more widely separated internal prosternal stripes, which are not joined in front.

\* *S. pastoralis* Duval (1852)

Marseul *Monogr.*, 1855, p. 463; Schmidt, *Best. Tab.*, 1885, p. 308; Reichardt, *Faune URSS*, 1941, p. 384.

S. France, Tunisia, Palestine, Turkmenia and Turkestan (after Reichardt *loc. cit.*), one specimen also from Salonica in Macedonia (leg. Schatzmayr!).

Eretz Israel: mouth of the Jordan, 25.III.97 (leg. Davydov!).

Subgenus *Chalcionellus* Reichardt

The position of *Saprinus mersinae* Mars., described by Sahlberg (1913) from Jericho, is uncertain. The species remained unknown to Reichardt, who mentioned it with a question-mark as *Chalcionellus* (Mitt. *Zool. Mus. Berlin*, 1932, p. 140). Its occurrence in Eretz Israel is very probable as the type, which was lost, originated from Syria. Solsky (*Nachr. Gesellschaft Naturf. XXX*, 1876, p. 239) also mentions Samarkand as a locality.

\* *S. blanchei* Marseul.

Marseul, *Monogr.*, 1855, p. 461; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 18, and *Faune URSS* 1941, p. 264 and 393; Müller, *Ent. Blätt.*, 1937, p. 110.

N. Africa, Near East, Central Asia; Macedonia (Salonica).

Eretz Israel: Bethlehem, 26.I.04 (Sahlberg 1913). I have seen a specimen from Ramat Gan, 24.III.45 (By.-S.), which exceptionally possesses a thin, engraved apical line at the end of the elytrae, whilst the posterior part of the sutural is shortened. The suborbital rounding of the head is first incurved, then elongated forward towards the clypeus.

\* *S. tyrius* Marseul

Marseul, *Monogr.*, 1857, p. 439; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 18, and *Faune URSS* 1941, p. 267 and 393.

Syria, Asia Minor, Caucasus, Turkmenia, Turkestan (Reichardt 1941). I know this species also from Salonica and Naxos (Schatzmayer), Crete (Paganetti), Cyprus (coll. By.-S.!), Baghdad (G. Frey) and Assur in Mesopotamia (Pietschmann).  
 Eretz Israel: Jericho, 12.III.04 (according to Sahlberg 1913).

*decemstriatus* Rossi (1792)

Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 86, and *Faune URSS*, 1941, p. 394; *conjugens* Payk. (1798); Marseul, *Monogr.*, 1855, p. 694; Schmidt, *Best. Tab.*, 1885, p. 311; Ganglbauer *Kaf. Mittelleur.*, 1899, p. 389.

Europe, Near East, Siberia, Turkestan; ssp. *tingitanus* Reichardt in N. Africa.  
 Israel: Carmel foothills, in coastal sands, 26.III.04 (Sahlberg 1913); Rehovot, 1.III.48 and Kfar Shmaryahu 13.III, few specimens (By.-S.!).

Subgenus *Zorius* Reichardt

*funereus* Schmidt

Schmidt, *Deutsche Ent. Zeitschr.*, 1890, p. 82; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 26, and *Faune URSS*, 1941, p. 394. *sublaevis* Sahlberg 1913, p. 17.

Hitherto known only from Israel: Schmidt's type originates from Haifa (ex. coll. Heyden); the same species was later found near Jerusalem (Ain Fara) by Sahlberg and described again as *sublaevis*.

Subgenus *Hypocacculus* Bickhardt

*S. praecox* Erichson (1834)

Schmidt, *Best. Tab.* 1885, p. 311; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 33, and *Faune URSS*, 1941, p. 396.

Canaries and Cap Verde Isles, N. Africa, Portugal, Sicily, Crete, Syria, Arabia, Ethiopia, Somaliland.

Israel: Haifa (Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 97).

*palaestinensis* Schmidt

Schmidt, *Deutsche Ent. Zeitschr.*, 1890, p. 86; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932, p. 34, and *Faune URSS*, 1941, p. 397. *spretulus* Marseul, *Monogr.*, 1885, p. 682 (nec 1862!); vide Peyerimhoff (*Bull. Soc. Entom. France* 1934 p. 261).

Algeria, Morocco. Israel: Nazareth (loc. class., coll. Schmidt).

*S. metallescens* Erichson (1834)

Marseul, *Monogr.*, 1855, p. 686; Schmidt, *Best. Tab.*, 1885, p. 310; Ganglbauer *Kaf. Mittelleur.*, 1899, p. 388; Reichardt, *Mitt. Zool. Mus. Berlin XVIII*, 1932 p. 35 and *Faune URSS*, 1941, p. 397.

Mediterranean, Arabia, E. Africa, Turkmenia, Turkestan.

Israel: Without exact location in coll. Rolph, and Haifa, coll. Schmidt (Reichardt, *op. cit.*, 1932, p. 106).

\* *S. baudii* Schmidt

Schmidt, *Deutsche Entomol. Zeitsch.*, 1890. p. 86; Reichardt *Mitt. Zool. Mus. Berlin* XVIII, 1932, p. 39, and *Faune URSS*, 1941, p. 398.

Described from Cyprus.

Israel: Haifa, coll. Schmidt (Reichardt, l. c. 1932, p. 112).

*S. Japhonis* Schmidt

Schmidt, *Deutsche Ent. Zeitschr.*, 1890, p. 83; Reichardt *Mitt. Zool. Mus. Berlin* XVIII, 1932, p. 44, 118, and *Faune URSS*, 1941, p. 399.

Israel: *loc. class.* Jaffa (coll. Schmidt). One specimen found also in Egypt (leg. Andres, *vide* Reichardt, l. c. 1932, p. 118).

Subgenus *Hypocaccus* Thomson

*S. apricarius* Erichson (1834).

Ganglbauer, *Käf. Mitteleur.*, 1899, p. 391; Reichardt, *Faune URSS.*, 1941, p. 31 and 402.

Mediterranean, Ethiopian and Indo-Malayan region, S. America; especially on sandy soil near seashore.

Israel: Meged, 20.IX; Raanana, 25.III; Ramat Gan, 24.III; Bat Yam, 10.IV; Rishon le Zion, 17.II; Nebi Rubin, 8.VIII; and Nir Arm, 1.X; in all places only single specimens (By.-S !). All specimens examined by me belong to the typical *apricarius* with punctate and microscopically charagrinate elytrae, and not to *rasilis* Mars., which Bickhardt *Arch. f. Naturgesch.*, 1921, p. 138) wrongly considers as a synonym of *apricarius*.

Gen. *Xenonychus* Wollaston

*X. tridens* Duval (1852)

Marseul, *Monogr.*, 1855, p. 501 and Schmidt, *Best. Tab.*, 1885, p. 309 (*Saprinus*); Ganglbauer *Käf. Mitteleur.*, 1899, p. 394; Reichardt *Faune URSS.*, 1941, p. 48.

Mediterranean.

Israel: sand dunes near Haifa, 5.IV.04 (Sahlberg 1913).

Gen. *Gnathoncus* Duval

*Gnathoncus rotundatus* Kug., quoted by Bodenheimer (1937) from Israel, requires revision, in view of the better characterization of species of this difficult genus achieved only recently.

Gen. *Tribalus* Erichson

\* *Tribalus scaphidiformis* Illiger (1807), mentioned by Sahlberg (1913) from Israel (Kishon river, 31.3.04), also requires revision, in my opinion, as this species mainly occurs in the W. Mediterranean. Hitherto, I have seen *Tribalus minimus* Rossi only from the E. Mediterranean (Trieste, Dalmatia, Albania, Corfu, Salonica, Rhodes and Aleppo).



Gen. *Platysoma* Leach

*Platysoma* (s. str.) cf. *algiricum* Lucas (1849)

Marseul *Monogr.*, 1853, p. 267; Schmidt *Best. Tab.* 1885, p. 285.

Algeria, Morocco, Sardinia, Sicily.

Eretz Israel: Gaza, 12.IV.41, and Hadera, 18.II.46 (By.-S. 2 specimens!). Both specimens resemble *Platysoma algiricum* from the W. Mediterranean in all their main characteristics; the same also applies to few specimens from Corfu (leg. Paganetti!) which deviate only slightly from the W. Mediterranean type. Small and not entirely constant differences in the stripes of the elytrae may probably justify the creation of an E. Mediterranean race of the *algiricum*, in which the three inner dorsal stripes are developed, at least in the apical half of the elytrae; in the specimens from Corfu the fourth dorsal stripe extends even as far as the basal third and is thus longer than the sutural stripe.

I assume that the specimens from Rhodes, which were classified by Schatzmayr, *coll. Zool. Gen. Agr. Portici XXXI*, 1941, p. 347, as *Platysoma simeani* Muls. et Godart, also belong to the E. Mediterranean form of *algiricum*. The true *P. simeani* would, according to the original description, possess only three teeth on the outer edge of all tibiae, whilst in the case of the specimens from Israel and Corfu, as well as in the case of the W. Mediterranean *algiricum*, at least the middle and posterior tibiae possess four teeth. According to Lewis, 1907, p. 342, *simeani* too has four teeth on the middle and posterior tibiae. With the aid of the types, it should first be determined whether *simeani*, *sensu* Lewis, is really identical with the species bearing the same name of Mulsant and Godart.

*Platysoma* (Cylistosoma) *cornix* Marseul

Marseul., *Monogr.* 1861, p. 153, Plate 3, Figure 13; Schmidt, *Best. Tab.*, 1885, p. 286.

S. E. Europe, Asia Minor.

Israel: Haifa, Carmel, 10.I; Ginegar, 3.III, below the bark of *Pinus halepensis*, not rare (By.-S.).

Gen. *Hister* Linné (s. lato)Subgenus *Macrolister* Lewis

*major* Linné (1766)

Marseul *Monogr.*, 1854, p. 173; Schmidt, *Best. Tab.* 1885, p. 288; Ganglbauer *zf. Mitteleurp.*, 1899, p. 361; Bickhardt, *Abh. Ver. Naturk. Cassel* 1919, p. 52.

Mediterranean.

Israel: Beersheba, 1.IV; Ruhama, 27.VIII; Nahalal, 6.II; (By.-S.).

Subgenus *Eucalohister* Reitt. (emend. Müller 1937)\* *H. kurdistanus* (Mars.) s. lato.

Embraces four forms which were described as separate species by Marseul: *kurdistanus* s. str. (*Monogr. Hist.*, 1857, p. 418) with all stripes complete on the elytrae, only the fifth pair being somewhat shortened at the basis; *scyta* (L'Abeille 1, 1864, p. 344) from Causasus and Mesopotamia, with greatly shortened fifth dorsal stripe, the four outer stripes and the sutural stripe complete; *lethierryi* from Algeria and *touthmosis* from Egypt (*Monogr. Hist.*, 1861, pp. 530 and 531) with greatly shortened fourth and fifth dorsal stripes, only the three outer stripes and the sutural stripe complete. *H. halepensis* from Syria, described by me (*Ent. Blätt.*, 1937, p. 122), is identical with *scyta* and has, therefore, to be included as a synonym.

Hitherto, only *kurdistanus* ab. *scyta* from Jericho was known for Eretz Israel (according to Sahlberg 1913). Owing to Bytinski-Salz, I have before me two specimens from Israel: one from Haluza (El Khalasa), 2.V.46! and a second one without exact locality!. Only the latter agrees very well with *scyta*, although the fourth dorsal stripe is very faint in the basal half and is reduced to small points on one elytrae; however, the outer lateral stripe on the pronotum is more strongly developed and is elongated backwards to reach the basal quarter. The other specimen, from Haluza, has a greatly shortened fourth dorsal stripe, exactly as in *lethierryi*, but differs either from it by the larger, circular and dense points on the propygidium; moreover, the outer lateral stripe on the pronotum is almost entirely obliterated, and even in the anterior corners is traceable by only a few dots. It remains to be seen whether this is an individual variant; only further collections in Israel may contribute to the clarification of the problem.

Subgenus *Hister* s. str.*H. uncinatus* Illiger (1807).

Erichson *Käf. Mark Brandenburg*, 1839, p. 662; *sinuatus* Illiger 1798 (nec Fabricius 1792); Marseul, *Monogr.*, 1854, p. 553; Schmidt *Best. Tab.*, 1885, p. 294; Ganglbauer *Käf. Mitteleur.* 1899, p. 364.

Europe, Mediterranean.

In Israel, represented by *reductus* ssp. nov. The diagnosis is: "*A forma typica europaea elytrorum striis duabus externis tantum integris, tertia postice fortiter abbreviata, ceteris internis omnino oblitteratis discrepans*". — The type originates from Jerusalem, 5.II.40; numerous paratypes from the same locality in March and April and Eilon, 27.III; Rosh Pina, 29.III, Mt. Carmel, 7.V; Nathanya 1.III; Kfar Shmaryahu 13.III.

*H. cadaverinus* Hoffmann (1803)

Marseul, *Monogr.*, 1854, p. 291; Ganglbauer, *Käf. Mitteleur.*, 1899, p. 363; Bickhardt, *Ent. Blätt.*, 1920, p. 99.

Europe, N. Asia.

Israel: Raanana, 25.III and 14.IV; Tel Aviv (By.-S.!).

Subgenus *Eudiplister* (Reitt., ex parte) Bickhardt

*E. castaneus* Men. (1832)

Reichardt, *Rev. Russe Entom.*, 1922, p. 52; *symrnaeus* Marseul, *Monogr. Hist.*, 1857, p. 308; Müller *Ent. Blätt.*, 1937, p. 124.

Described by Menetries from Caucasus, by Marseul from Asia Minor; according to Schmidt (1885) also in Greece. My collection contains one specimen from Armenia (Ordubad, leg. Kulzer).

Israel: Jerusalem 7.III and Deir Aban E. Judean Mountains 29.II (Sahlberg 1913). I possess one specimen from Haifa (Reitter), which corresponds exactly to the original description of *symrnaeus*; slightly deviating is a specimen from Tel Aviv 11.III.47 (By.-S.!) identical with *symrnaeus* as regards body shape and the almost parallel lateral prosternal stripes of the pronotum, which are also widely separated in front of the basis, but with two very thin, prosternal stripes between the fore coxae, which are very greatly shortened cranially.

*E. peyronei* Marseul

Marseul, *Monogr.*, 1857, p. 420; Reichardt, *Rev. Russe Entom.*, 1922, p. 52; Müller, *Entom. Blätt.*, 1937, p. 124.

According to Marseul this originates from the same region as *symrnaeus*, i.e. Asia Minor; according to Bickhardt *Genera Insectorum (Histeridae)* 1917, p. 188, also Syria and Turkestan. In my collection are also a few specimens from Afghanistan (Kuschke).

A specimen collected by Dr. Bytinski-Salz near Nir Am, 21.III.46! also seems to belong to this species. It differs from *symrnaeus* by the slightly greater breadth of its body and by the lateral stripes on the pronotum, which are only narrowly separated in front of the base, exactly as with the genuine *peyronei*; however, the usual thin prosternal stripes of the latter are missing. It is probable that the development of the prosternal stripes of *peyronei* as well as of *symrnaeus* is a variable feature and cannot be used as a criterion of the species, as I assumed previously (*Ent. Blätt.* 1937). In any case, the examination of more material from Israel would be necessary to clarify this problem fully.

Subgenus *Merohister* Reitter

*E. ariasi* Marseul

Marseul, *L'Abeille* I, 1864, p. 342; Schmidt, *Best. Tab.*, 1885, p. 468; Reitter, *Monogr. Germ. II.*, 1909, p. 282. Mediterranean (Spain, S. France, Italy, Trieste, Asia Minor).

Israel: Pardess Hannah, VI.46 (By.-S. 1 specimen!).

Subgenus *Paralister* Bickhardt

*E. graecus* Brullé 1829

Marseul *Monogr.*, 1854, p. 529; Schmidt, *Best. Tab.*, 1885, p. 292; Ganglbauer, *Öf. Mitteleur.*, 1899, p. 366; Müller, *Entom. Blätt.*, 1937, p. 125 (races).



The type race in Greece, Syria and Israel; ssp. *cyrenaicus* m. in Libya, ssp. *horni* Bickhardt in Algeria, ssp. *mauritanicus* m. in Morocco.

Israel: Judea and Galilee, II-III (Sahlberg 1913); Kiryat Anavim, XII (Bodenheimer 1937); Jerusalem, 16.II-30.III; Bab el Wad, 3.I; Ben Shemen, 20.IX; Eilon, 9.III; Naharia, 15.V; Benyamina, 16.III-17.IX; Haifa, 4.XII (By.-S.). All specimens I examined belong to the type, without suture.

\* *H. carbonarius* Illig. (1798)

Marseul, *Monogr.*, 1854, p. 534; Schmidt, *Best. Tab.*, 1885, p. 293; Ganglbauer, *Käf. Mitteleur.*, 1899, p. 367; Müller, *Ent. Blätt.*, 1908, p. 121.

Europe, Asia Minor.

Owing to Dr. Bytinski-Salz, I saw a few specimens from Israel: Tel Aviv, 2.III and Rehovot, 15.XI.47!. These specimens as well as those from the Vardar V.46.6 and Istanbul ! possess a coarser punctuation on the propygidium than the Central European specimens and thus belong to *macedonius* m. (*Ent. Blätt.* 1937, p. 127).

*H. stercocarius* Hoffmann (1803)

Widely distributed in Europe; also in Asia Minor (Pisidian and Karischian Taurus) and Turkestan (Issyk Kul!).

According to Bodenheimer (1937) also in Eretz Israel.

Subgenus *Grammostethus* Lewis

\* *H. ruficornis* Grimm (1852)

Schmidt, *Best. Tab.*, 1885, p. 292; Ganglbauer, *Käf. Mitteleur.*, 1899, p. 367; Reitter, *Fauna Germ.*, 1909, p. 285.

Central Europe, Asia Minor.

Israel: Tel Aviv, 31.X, 1 specimen (By.-S.).

Subgenus *Peranus* Lewis

*H. scutellaria* Erichson (1834)

Marseul, *Monogr.*, 1854, p. 579; veris. *scutellaris* auct. ex parte (? exclus. *lentus* Mars.).

E. Mediterranean, S. Italy (M. Gargano) and Sicily.

Eretz Israel: the uniformly black type from Nablus (Reiche et Saulcy, *Ann. Soc. Ent. France*, 1856, p. 367, quoted as *foveicollis* i. litt.); 1 specimen also from Benyamina, 7.VI (By.-S.).

Remark: Most authors name the uniformly black type of *scutellaris* as *lentus* Mars. It is, in my opinion, questionable whether the real *lentus*, described from Senegal, is only a colour variation of *scutellaris*. One specimen from W. Africa (French Guinea) in my collection exactly corresponds to the description and picture of *lentus*, but differs from the E. Adriatican specimens of *scutellaris* by the inner lateral

stripe, which is *not* interrupted anterior to the pronotal depression, by the longer and most complete sutural stripe and by the more intense punctation on the propygidium. The taxonomical value of these differences can be decided upon only by the study of more material from W. Africa.

### Subgenus *Atholus* Thomson

#### *duodecimstriatus* Schrank (1781)

Marseul, *Monogr.*, 1854, p. 586; Schmidt, *Best. Tab.* 1885, p. 295; Ganglbauer, *cf. Mitteleur.*, 1899, p. 369.

Europe and Mediterranean.

The statement "Siberia and Japan" in Junk's catalogue, 1910, and in Wytzman, 1917, has to be examined. Specimens in my possession from Turkestan, Siberia and Japan differ from the European *duodecimstriatus* in their mandibles, which are less strongly excavate and thus have a blunt outer edge.

Eretz Israel: Jericho, 28.II.1894 (Sahlberg 1902-03); Judea and Galilee, 24.II-29.III (Sahlberg 1913). Also: Jerusalem, 16.III and Ma'ale Hahamisha, 28.V (By.-S!).

#### *H. corvinus* Germar (1817)

Marseul, *Monogr.*, 1854, p. 588; Schmidt, *Best. Tab.*, 1885, p. 296; Ganglbauer, *cf. Mitteleur.*, 1899, p. 369.

Europe, Syria.

Israel: Jerusalem, 24.II (Sahlberg 1913).

#### *bimaculatus* Linné (1758).

Marseul, *Monogr.*, 1854, p. 582; Schmidt, *Best. Tab.*, 1885, p. 296; Ganglbauer, *cf. Mitteleur.*, 1899, p. 369.

Europe, Mediterranean; Eritrea (Asmara! and Omager!); E. India (Madras!, Mysore! and Karikal Territory!).

Israel: Deir Aban, W. Judean mountains, 29.II, in horse dung (Sahlberg 1913); Iron, 31.VII; Gat, 17.VI, (By.-S., 8 specimens!).

### Gen. *Spatochus* Marseul

#### *coyei* Marseul

Marseul, *L'Abeille.*, 1864, p. 341.

Described from Syria and Asia Minor (Smyrna). Also in Caucasus (Elisabetpol!) and Arabia.

Israel: Collected near Jerusalem by Pic, together with *Myrmecocytsus viaticus*

var. *niger* Andre (vide *Bull. Soc. Ent. France*, 1900, p. 171). As Pic's short note says nothing about the colouration of the specimens collected by him, I assume it to be the normal, red-brown type. On the other hand, Reitter described an entirely black variety also from Jerusalem, which he called var. *nigrinus* and which is said to have been found by Leuthner in March 1885, with a *Lasius* species (vide *Wien. Ent. Zeitung* 1906, p. 32).

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## THE TICKS OF SINAI\*

B. FELDMAN-MUHSAM

*Department of Parasitology, The Hebrew University of Jerusalem*

### ABSTRACT

Ticks from Sinai taken from domestic stock and wild animals, mostly rodents, were examined. They included specimens of the cosmopolitan species *Rhipicephalus sanguineus*; and of *Hyalomma dromedarii* and *H. excavatum* which are widely spread throughout the Middle East. In addition, representatives of the tick fauna of Asia, Africa and Europe were encountered. *R. rossicus* was recorded for the first time from the Middle East. The male and the female of a new species, *H. sinaiti* are described.

The Sinai peninsula serves as a bridge between Asia and Africa and its fauna is therefore of special interest. Since ancient times caravans have been passing through, on their way between the two continents and have probably played a major part in introducing parasites into the area.

As the fauna of the two continents is quite different it is interesting to observe, among other things, the transition of the tick fauna from one continent to the other. It is surprising that although more than 50 species of *Rhipicephalus* ticks are known from the African continent, of which 18 species are from Sudan, according to Hoogstraal (1954), only *R. sanguineus* s.l. is enzootic in Egypt (Hoogstraal and Kaiser, 1958). Three east African species have been introduced but not established here. In Israel, the nearest country in Asia to Africa, in addition to *R. sanguineus*, s.str., *R. secundus* and *R. bursa* have been recorded.

The study of the Sinai tick fauna is therefore interesting not only *per se*, but from the developmental and zoogeographical aspects as well.

### MATERIAL EXAMINED

*Rhipicephalus sanguineus* (Latreille, 1806)

♂♂ + ♀♀ from dog, domestic cat, goat, *Lepus*, *Vulpes*, *Paraechinus dorsalis*, on ground. Nymphs from *Acomys*.

ST. CATHERINE'S MONASTERY, FEIRAN OASIS, WADI FEIRAN.

It should be emphasized that we refer here to *R. sanguineus* s.str. (Feldman-Muhsam 1952). *R. secundus* Feldman-Muhsam, 1952, has not been found among the material from Sinai. This is most surprising as this species has been diagnosed by us both

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in Israel and Egypt (unpublished). As a considerable number of ticks (29 ♀♀) were taken from goats, and in Israel this species accounts for 91 % of the *sanguineus* population on goats (Feldman-Muhsam 1956), we would have expected to find at least some *R. secundus*.

*Rhipicephalus rossicus* Yakimoff and Kohl-Yakimoff, 1911

1 ♂ from goat.

ST. CATHERINE'S MONASTERY.

Since its description the species has not been mentioned outside Russia. Orlov recently Serdyukova (1956) gives the geographical distribution as Ukraine, northern Kazakhstan, Bulgaria and the Turkish-Armenian border.

This seems to be the first report of the species since the above mentioned one. We have also diagnosed several specimens from Israel (unpublished).

*Haemaphysalis* sp.

1 nymph from *Paraechinus dorsalis*.

ST. CATHERINE'S MONASTERY.

*Hyalomma schulzei* Olenov, 1931

♂♂ + ♀♀ from camel, on ground.

Nymphs from *Gerbillus calurus* and *Meriones crassus*.

ST. CATHERINE'S MONASTERY, FEIRAN OASIS, WADI MUKATTAB.

This species was described for the first time from northern Iran (Olenov, 1931) but later proved to be present also in Afghanistan (Anastos, 1954), southern Israel which is a semidesert area (Adler and Feldman-Muhsam, 1948) and Egypt (unpublished).

*H. schulzei* is most probably of an Asiatic origin and may have been carried by camel caravans through southern Israel into Sinai and Egypt.

*Hyalomma dromedarii* K., 1844

♂♂ + ♀♀ from camel, on ground

Larvae from jerboa, gerbil.

ST. CATHERINE'S MONASTERY, ARAIF EN-NAKA, BIR SAWRA, WADI FEIRAN, FEIRAN OASIS, DAHA WADI SUDR.

Koch records the species from Egypt and Asia Minor.

It is difficult to determine as yet the origin of this species. *H. dromedarii* has undoubtedly migrated with camel caravans through a wide area in southwestern Asia (including Afghanistan, Pakistan, Iran, Iraq, Jordan, Israel and Sinai), the whole northern third of Africa to the western coast of Africa, as far as the Canary Islands as well as in the opposite direction.

*Hyalomma excavatum* K., 1844

♂♂ + ♀♀ from camel, goat.

Larvae from *Meriones crassus*, *Gerbillus calurus*, *Dipodillus*.

Nymphs from *Lepus*.

ST. CATHERINE'S MONASTERY, WADI FEIRAN.

The geographical distribution of *H. excavatum* corresponds approximately to that of *H. dromedarii*. The most common hosts of this species in the Middle East are cattle and camels. The continuity of the area of its distribution suggests that camels may play an important role in the spread of this species.

*Hyalomma rufipes* K., 1844

♂♂ + ♀♀ from camel, goat.

1 nymph from *Acomys dimidiatus*.

ST. CATHERINE'S MONASTERY, WADI FEIRAN.

This species is prevalent in South Africa. We have determined lots from Egypt and Sudan off cattle. In Israel *H. rufipes* is more frequent in the southern semi-arid parts of the country than in the north, mainly on camels. Various Russian authors (Olenev, 1931; Pomeranzev, 1950) recorded the species under different names (*H. aequipunctatum*, *plumbeum*, *impressum*, etc.)

The range of distribution of this species does not suggest that the camel would play a major role in its dissemination. In this species this role is no doubt performed by birds. Hoogstraal and Kaiser (1958) have reared 220 adults of *H. rufipes* from nymphs secured from passerine birds in Cairo during their spring migration to Europe.

*Hyalomma marginatum* K., 1844

2 ♂♂ from camel.

3 nymphs from *Lepus*.

WADI FEIRAN, ST. CATHERINE'S MONASTERY.

*H. marginatum* is a southern European species, which has spread on one side to western North Africa through Gibraltar, and on the other side to Turkey, Israel and sporadically to Sinai and down to Egypt.

*Hyalomma impeltatum* Schulze and Schlottke, 1929

Larva from *Acomys dimidiatus*.

Nymphs from *Gerbillus calurus* and *Lepus*.

ST. CATHERINE'S MONASTERY.

This species was first described by Schulze and Schlottke in 1929, and by Delpy (1946) as *H. brumpti*.



The synonymy of *H. brumpti* with *impeltatum* was established by us in 1954.

The geographical distribution of the species is still poorly known. Delpy recorded the species from Iran and Cameroon and Kratz from Rio de Oro.

Since the species was unsatisfactorily described, it must have probably been diagnosed under a number of specific names.

We have determined lots from Sudan off cattle, camels and horses. According to Hoogstraal and Kaiser (1958) it is very widely distributed in Egypt. In Israel it is quite a rare species and was collected mainly in the south, from camels.

More records are badly needed in order to estimate the zoogeography of the species.

### *Hyalomma sinaii* n. sp.

1 ♂ + 3 ♀♀ from goat.

ST. CATHERINE'S MONASTERY, May 1953.

♂ holotype and ♀ allotype in my own collection.

**Male:** The scutum is oval and narrow  $3.25 \times 2$  mm; light red brown. Middle of scutum smooth, with a few large and superficial dots. Caudal area with many medium-sized and very few large dots. Cervical grooves long, deep and wide; they extend posteriorly and superficially to the level of the stigma. Marginal grooves short (as in *excavatum*). They are continued anteriorly almost to the level of the eyes in a line of a few very large and well separated dots. Median groove is narrow and long, paramedians wide (figure 1,A). Parma somewhat lighter than the other festoons. Lateral and posterior edge of anal plate forms a single smoothly convex line. Subanal plates are very small. Adanal plates quite large and rounded, (figure 1,C). Stigma retort-shaped. The body of the stigma is very small and the tail long and narrow (figure 1,D) but relative to the body of the stigma the tail is wide. Basis capituli has obtuse lateral angles. The palps, as compared with those of *H. excavatum*, are long and narrow (figure 1,B). Legs yellowish without dark rings. Only the fourth pair has a hint of brown annulation.

**Female.** Scutum light red brown as in the male, with some medium-sized dots intermingled with finer dots. The scutum is as long as wide (ca. 1.5 mm).

Cervical and lateral grooves form a deep and large furrow which reaches the posterior edge of the scutum (figure 2,A). Basis capituli hexagonal (figure 2,B) with obtuse lateral angles (these are less obtuse and less protruding than in *Hyalomma minna*). Cornua small and wide.

Palps long and narrow. Article II — yellow, III — yellow-brown. Legs yellowish with white annulation. Legs III and IV with a dorsal longitudinal white stripe. The entrance into the vaginal tube is not covered by an operculum, and the area within the "cup" is concave when unmounted. The mounted genital aperture (figure 3) is like a deep cup, and the flaps wide and do not taper.

**Discussion:** We think that these ticks represent a species, not previously

described. Nevertheless, we hesitated to describe it as a new species, because, on the one hand, we are aware of the extreme variation existing within most species of *Hyalomma*, and, on the other hand, because of the probability that these specimens might belong to one of the many "new species" of *Hyalomma* described during the last 50 years.

In addition, in studying collected material, one can never be sure that a male and a female from the same lot belong actually to the same species. But as our male and females resemble each other in size, punctuation and colour, it may be assumed that they represent one species.

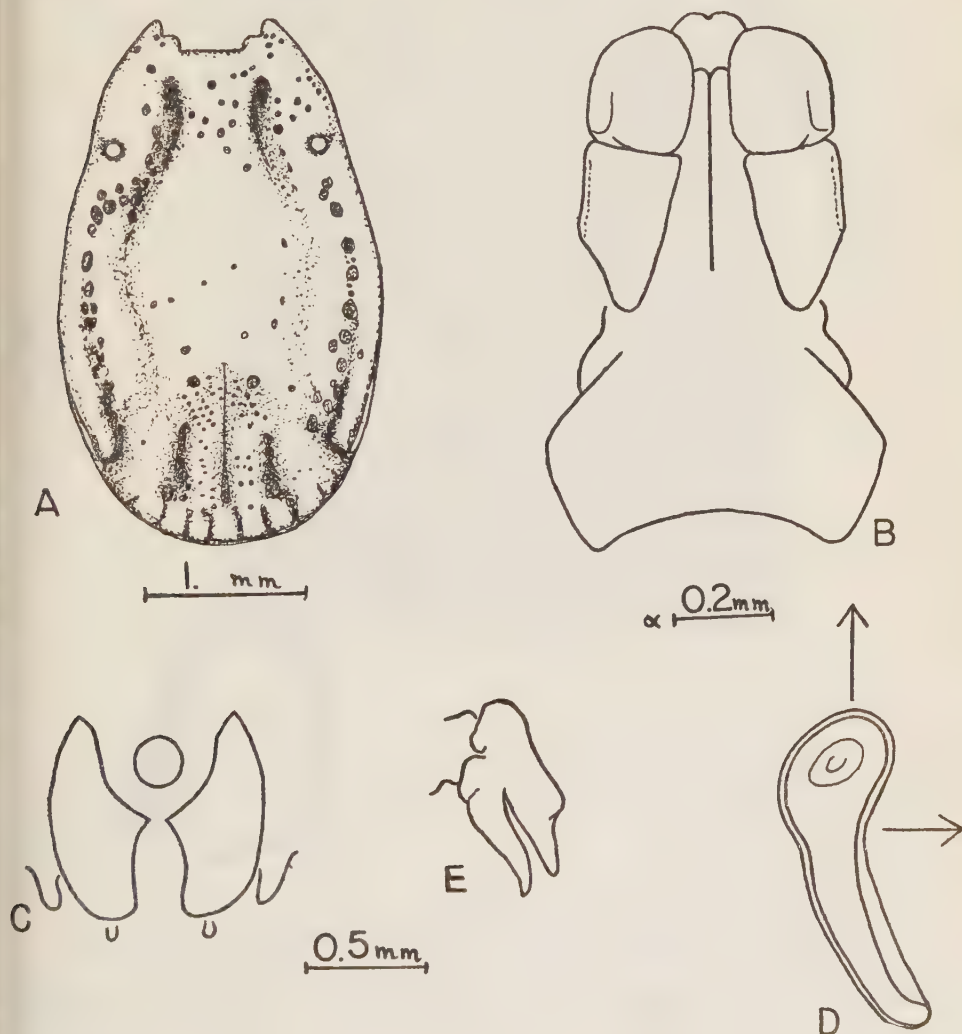


Figure 1

*Hyalomma sinaii* n. sp. Male. A — Scutum, B — Capitulum, C — Anal armature, D — Stigma, E — First coxa. Scale a — B, D

Following Kratz's key, which covers all species known at his time and accepted by Schulze and his collaborators, our specimens could perhaps be *H. excavatum*, *H. iberum* Sch. and Schlottke, 1929, or *H. fezzanensis* Tonelli-Rondelli, 1935.

The male might fall within the range of variation of *H. excavatum*, but not the female.

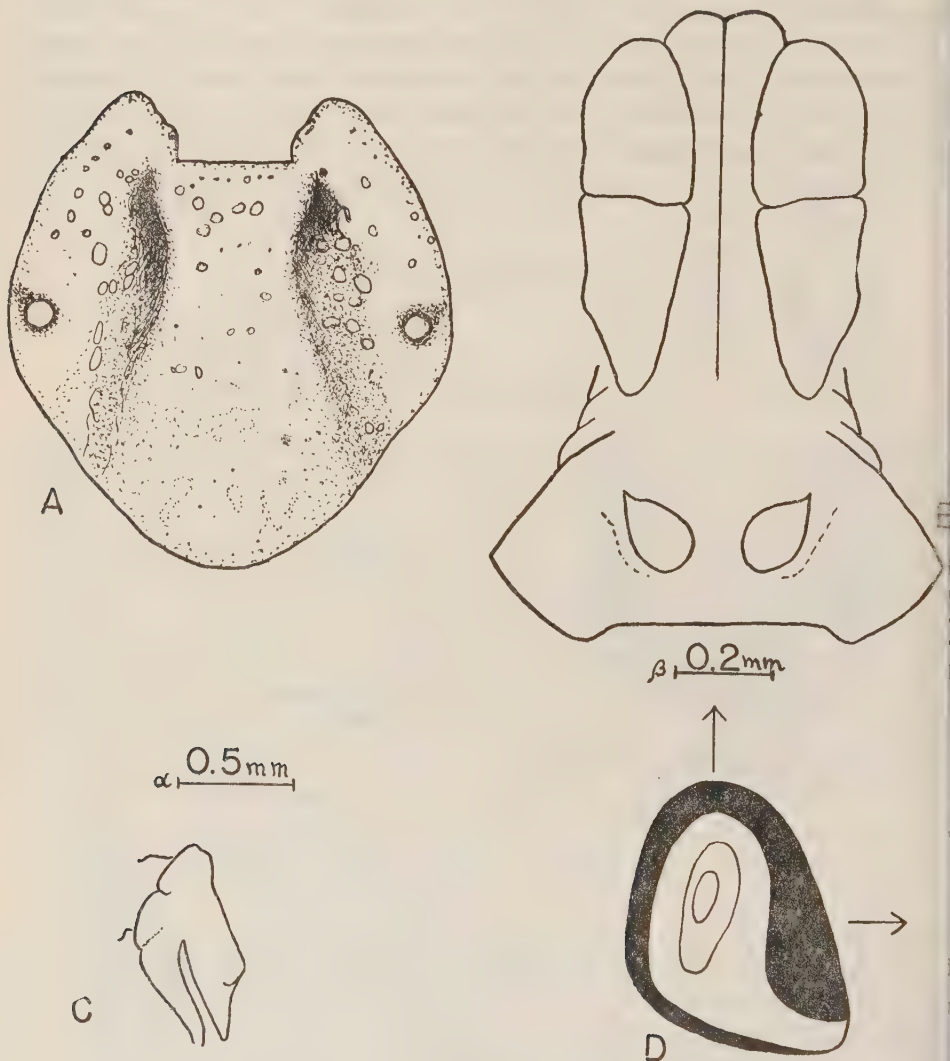


Figure 2

*Hyalomma sinaii* n. sp. Female. A — Scutum, B — Capitulum, C — First coxa  
D — Stigma. Scale  $\alpha$  — A, C; Scale  $\beta$  — B, D

The form of the mounted genital aperture does not leave any doubt that at least the females are not *H. excavatum*.

According to Kratz's key, our male specimen might have been *H. iberum* because of the unusually small main part of the stigma and its very narrow tail. But the exam-



nation of the type specimen of *H. iberum* does not show the characteristics which Kratz ascribes to *H. iberum*. (For an unknown reason he does not depict the type specimen).

Kratz's key suggests also the possibility of identity with *H. fezzanensis* Tonelli-Rondelli, 1935, but the examination of the female type specimen of *H. fezzanensis*



Figure 3

*Hyalomma sinaii* n. sp. Female. Mounted genital aperture.  $\times 200$

(kindly lent to us by Dr. Delfa Guiglia from the Museum of Genoa), shows that it is not the same species. In *H. fezzanensis* the scutal dots are more or less of medium and equal size, whereas in our specimens the dots are very fine, with only a few larger ones.

The unmounted genital aperture of *H. fezzanensis* is V-shaped and the area in the V is bulging but in addition the two branches of the V are bordered with two lip-like bulges. This last characteristic together with the punctation of the scutum definitely puts *H. fezzanensis* in synonymy with *H. impeltatum* (and not with *H. dromedarii* as stated by Tendeiro, 1955).

The male type of *H. fezzanensis* seems to be lost; no comment can therefore be given on it.

Our specimens, as compared to the above mentioned species, showed marked differences in the form of the female genital aperture (mounted and unmounted), the shape of the basis capituli and palps in both sexes and of the stigma of the males. We therefore feel justified in establishing *H. sinaii* as a new species.

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## PARTURITION IN SCORPIONS

A. SHULOV, R. ROSIN AND P. AMITAI

*Department of Zoology, The Hebrew University of Jerusalem*

### ABSTRACT

Observations have been carried out on parturition of *Nebo hierochonticus* E. Sim. (Diplocentridae), *Orthochirus innesi* E. Sim., *Leiurus quinquestriatus* H. et E. and *Compsobuthus acutecarinatus* E. Sim. It has been found that *Nebo* is viviparous and *Orthochirus*, *Leiurus* and *Compsobuthus* are ovoviviparous.

Very little has been known of the biology of scorpions until quite recently.

It is only four years since observations were made on the process of mating of scorpions, almost simultaneously in Germany (Angermann 1955), South Africa (Alexander 1956), South America (Zolessi 1956) and Israel (Shulov and Amitai 1956).

It has been suggested that scorpions are either viviparous or oviparous or both (Millot and Vachon 1949, in Grassé).

Shultze (1927), cited by Angermann (1957), was apparently the first who briefly mentioned the process of parturition in *Palmanus longimanus*. Angermann (1957) gave a description of parturition in *Euscorpius italicus* (Chactidae).

We have had the opportunity to witness the act of parturition on several occasions in the following four species:

*Nebo hierochonticus* E. Sim. (Diplocentridae).

*Orthochirus innesi* E. Sim. (Buthidae).

*Leiurus quinquestriatus* H. et E. (Buthidae).

*Compsobuthus acutecarinatus* E. Sim. (Buthidae).

The following is a brief report of the process; a detailed description will be published elsewhere.

The parturition of *Nebo hierochonticus* has been observed to proceed as follows:

The mother, a specimen of about 10 cm in length, was obtained in the hill area of Tel-Yerucham (Northern Negev) on the 30th of July 1959. She was found to be very much swollen and inside her abdomen blurred spots were visible, which, in the course of time, acquired a more distinct shape. A month later the embryos, appearing as ellipses lying in an oblique position, could be easily distinguished through the lateral wall of the female's abdomen, especially at the posterior half of the mesosoma. A few days later the black eye-dots and body segmentation of the embryos could be easily discerned.

Parturition began on the 8th of September 1959 and lasted three days (8.IX.59-



10.IX.59). The number of newly born scorpions was 45. The emergence was in groups with intervals lasting up to a few hours. The mother, which was kept in a dimly lit room, was observed to give birth during the night as well as during the day.

No visible contraction of the body muscles was seen during parturition; on the other hand, peculiar movements of the tail were observed, which are undoubtedly related to the act of parturition. These movements consisted of slight and/or vigorous thrusts of the tail backwards, sometimes accompanied by tilting its end to one side or waving it from one side to another. Occasionally the tail was stretched fully back until it lay flat on the ground but soon resumed its original position of being slightly arched over the body.

These movements of the tail are similar to those adopted by the male of the same species during ejection of the spermatophore, the main difference being that the female's thrusts do not assume the very rapid rhythm performed by the male, and do not exhibit any regularity. The thrusts were sometimes observed even a few hours before the actual emergence of the young, whereas during the act of emergence such movements were not always seen; in some cases these movements were accompanied by elevation of the pedipalps.

During the act of parturition the mother assumed a particular posture. The anterior part of her body was raised some 1.5 cm above the ground, the posterior part of the mesosoma as well as the pedipalps were resting on the ground. The pectines were pointing obliquely downwards, forming an angle of about 45° with the body's axis and remained motionless.

Before the beginning of parturition the mother would scratch the ground with the first or second pair of legs, or both. Later she would fold the first pair at the patellar and tibial joint and rest them on the ground in front of the genital aperture, in such a position that tibia and tarsus of one leg were stretched either along those of the opposite one, or else practically on top of it. The second and third pair would also rest on the ground around the area which lies exactly beneath the genital aperture. Thus the group of newly born scorpions which gradually aggregated under her body was surrounded by a sort of basket formed by the mother's legs. This position of the legs seems to protect the young and hinder their straying away from the mother.

In all six cases in which the emergence of single individuals was observed in detail they were seen to emerge with their tails first, then came the body and legs, the head and pedipalps emerging last. (In another case of parturition of *Nebo hierochontica* the neonati were observed to emerge mostly in pairs, one with the head first, the other with the tail first).

The emergence of a single young lasts 10 minutes on the average. The newly born scorpions are able to move even before they are completely detached from the mother's body. In one case a young scorpion was observed to twist vigorously the posterior half of its body, while the anterior half was still inside the body of its mother.

The young do not undergo any moult shortly after birth (the first moult occurring some ten days after birth). They begin to climb on the mother's legs and pedipalps

and subsequently on her back almost as soon as they are detached from her body. This feat is accomplished very slowly and gradually, the little scorpion performing a few crawling movements, sometimes without making any real advance at all, then resting for five to fifteen minutes, after which it resumes its crawling. It takes the young scorpions about an hour to reach their mother's back. The mother does not in any way assist or direct them in their climb, but it should be noted that due to the special posture assumed by her legs, as mentioned above, the young can hardly avoid coming in contact with her legs.

When a newly born scorpion is laid on its back it will turn over unaided, though it seems not to be disturbed by lying upside down, and may remain quite motionless in this position for some time.

The mother exhibits marked "nervousness" during parturition, twisting swiftly in the direction of any of her young which might have fallen from her back, or are crawling on the ground beside her. Often she may catch one of these, pass it between her pincers, hold it for some time and lay it down apparently causing it no harm at all. Occasionally she will raise it toward her mouth and on one occasion, when left unhindered, the mother actually ate one of her young.

Apart from cannibal traits, the mother generally showed during the period of parturition a marked appetite. She was actually observed to hold bits of food in her "mouth" while the young were emerging, but she was not chewing them.

A female of *Scorpio maurus fuscus* (Scorpionidae) was found in our collection preserved in alcohol, with a young larva almost fully emerged from the female's body (see photograph). In this case, the larva, like those of *N. hierochonticus*, was free from any foetal envelope.



1. *Compsobuthus acutecarinatus* in the act of parturition.

Observations on parturition of scorpions of the Buthidae family show some differences from those of *N. hierochonticus* (Diplocentridae).

The parturition of *Orthochirus innesi* was observed on the 16th of July 1958, when the mother was discovered in the middle of the delivery. The mother was stanced firmly on her posterior pair of legs, the anterior pair were slightly bent and turned inward. The posterior part of the mesosoma rested on the ground, cephalothorax and anterior part of the mesosoma were raised forming an angle of  $30^\circ$  with the ground. The genital aperture opened and a white object emerged slowly. This process was accompanied by a slight trembling of the body and raising of the mother's tail (in form of "L'arbre droit").

It takes one young scorpion some 4 minutes to complete its emergence. The young scorpions emerge with head first, the tail resting on the ventral surface of the mesosoma. The body, tail and appendages of the neonatus are enveloped together in a very thin, transparent and fragile foetal envelope.

About one minute after its emergence, the little scorpion begins to twist its body and the foetal envelope disintegrates into minute, almost microscopic fragments. Some 8-10 minutes later the young larva begins its first moult. When parturition is completed the mother lowers the anterior part of her abdomen over the group of young twisting on the ground, at the same time surrounding them with her pedipalps and the larvae which have already succeeded in struggling out of the first real larval skin begin to climb on to their mother's back.



2. *Scorpio maurus* with a neonatus emerging.



Parturition in *Compsobuthus acutecarinatus* and *Leiurus quinquestriatus* is similar to that observed for *Orthochirus innesi*. The young scorpions also emerge in their foetal envelopes which quickly disintegrate into minute particles. The first moult occurs during the first hour after parturition, and only then the young begin to climb onto their mother's back.

Angermann (1957) mentions only the foetal envelope from which the newly born scorpions emerge before climbing on the mother's back, but he did not observe the first moult within this period.

The parturition in Diplocentridae and Buthidae differ slightly in the posture and behaviour of the mother. However, there is a very marked difference in the condition in which the young scorpions are born. Diplocentridae (apparently Scorpionidae as well) are viviparous, the young are born free of any envelope and there is no moult immediately following their birth, whereas in Buthidae they emerge in foetal envelopes and begin to climb on their mother's back as soon as they have broken out of the foetal envelope and have gone through their first moult. Thus it may be concluded that the Buthidae observed are ovoviviparous, whereas *Nebo* (Diplocentridae) and *Scorpio* (Scorpionidae) are viviparous and *Euscorpius* (Chactidae) being an intermediate type.

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